ADHERENS JUNCTIONS ORGANIZE THE PANCREATIC DEVELOPMENTAL PROGRAM AND MANAGE THE EVOLUTION OF NEOPLASTIC EPITHELIUM

by
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A dissertation submitted to Johns Hopkins University in conformity with the requirements for the degree of Doctor of Philosophy

Baltimore, Maryland
February, 2016

ABSTRACT

The intracellular protein p120 catenin aids in maintenance of cell-cell adhesion by regulating E-cadherin stability in adherens junctions. Recent studies have shown essential roles for the cell-cell adhesion apparatus during development and in several human disease states, which has precipitated a rapidly growing interest in the translational biology of these molecules. The work described herein advances our knowledge on the function of adherens junctions during mammalian development and disease states such as injury and neoplastic progression.

Chapter 1 provides an overview of established biology of adherens junctions in mammalian development, physiology, and cancer. In chapter 2, we identify a crucial role for p120 catenin in pancreatic development to include regulation of tubulogenesis, branching morphogenesis, acinar cell differentiation, migration of endocrine clusters, and actin cytoskeletal organization. Chapter 2 also reports a critical function for p120 catenin in regulation of inflammation in neonatal and adult mouse pancreas.

Chapter 3 explores the biology of p120 catenin in pancreatic disease states to include early pancreatic neoplasia and experimental pancreatitis. Based on literature and *in vivo* evidence linking p120 catenin to both tumor suppressor and metastasis promoting activities in various tissue contexts, a conditional mouse model approach was used to examine the function of p120 catenin in premalignant pancreatic cancer. Surprisingly, a remarkable epithelial cell delamination (also known as basal epithelial cell extrusion) phenotype was unmasked in the context of acinar-cell specific deletion of p120 catenin and activation of oncogenic Kras in adult mice. This was accompanied by a strong desmoplastic response and significant immune cell infiltration. This mouse model

unveiled a new mechanism for pancreatic neoplastic epithelial cell invasion. We identify S1P/S1pr2 as the key mediator of increased basal epithelial cell extrusion in the context of p120 catenin loss in an *in vitro* system. Chapter 4 presents a brief summary of how the data described herein advances the field. Together, these results establish a completely novel role for p120 catenin in regulation of basal epithelial cell extrusion and invasion.

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Co-mentor: Jennifer M. Bailey, MA, PhD

Thesis readers: Steven D. Leach, M.D and Andrew J. Ewald, PhD

PREFACE

Above all, I'd like to share how fortunate I have been to have the opportunity to work with and learn from my advisor, Steven D. Leach and co-mentor, Jennifer M. Bailey. Steve's ardor for science and ambition to push beyond frontiers is paralleled by his prudent approach to scientific questions. Jennifer's excellent, scientific aptitude has brought her to the forefront of discovery where she has integrated new techniques and enabled scientific breakthroughs by virtue of careful and thoughtful design. Thank you both for demonstrating how leaders inspire and for developing and shaping my scientific faculty.

I would also like to acknowledge all the members of the Leach and Bailey labs, past and present, who have guided, promoted, and nurtured my intellectual and personal growth. I am particularly grateful to Elayne Provost, Janivette Alsina, Kelly J. Lafaro, Florencia McAllister, Yue J. Wang, Megan H. Cleveland, and Meritxell Rovira Clusellas for expanding my repertoire of experimental techniques and support of my scientific endeavors.

I would like to give recognition to the financial, educational, and administrative support of the Institute of Genetic Medicine Human Genetics program and Department of Surgery at Johns Hopkins University School of Medicine as well as the Department of Internal Medicine, Division of Gastroenterology, Hepatology, and Nutrition at UT Health Science Center at Houston. They have engendered an enriched community of learning and scientific exploration and created a home for me in both Baltimore and Houston.

I would be remiss if I did not acknowledge the members of my thesis committee, Andrew J. Ewald, Christine A. Iacobuzio-Donahue, Anirban Maitra, Jennifer M. Bailey, and Steven D. Leach who have provided substantial intellectual contributions and guidance towards the genesis and direction of my scientific undertakings and career. I am especially thankful for Jennifer M. Bailey's support of a unique and invaluable experience in Houston and Andrew J. Ewald's helpful advice.

Most importantly, I cannot begin to express how happy I am to have devoted and compassionate parents and family who have wholeheartedly supported this journey. From my affectionate grandmother, who gave all her strength and vitality to her family to my gifted brother Dean, who constantly surprises and impresses me with his talent and achievements, I am surrounded by motivation. Mom and dad, your commitment to helping your children succeed and triumph any obstacle is unmatched. I can tell you with complete certainty that I would not have started this path without your unending dedication and encouragement, which has served as steadfast inspiration.

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CHAPTER 1 – INTRODUCTION

Adherens junctions

Adherens junctions are one of several transmembrane junctional complexes that mediate cell adhesion in polarized epithelial cells (Figure 1.1). The function of adherens junctions are to link the actin cytoskeleton of neighboring cells via cadherins and adapter proteins like catenins and vinculin. Adherens junction proteins serve roles in tissue homeostasis, embryonic development, tissue morphogenesis, and tumorigenesis (Hartsock & Nelson, 2008; Perez-Moreno & Fuchs, 2006). Accordingly, cadherins and catenins have been the subject of intense research and interest since their discovery in the 1970s and 1980s, respectively (Yap, Gomez, & Parton, 2015).

Epithelial integrity is essential for proper organogenesis during development and tissue homeostasis. Adherens junctions are an integral part of the maintenance of tight cell-cell adhesion in epithelial tissues. The core of the adherens junction in epithelial tissues is comprised of E-cadherin, β-catenin, p120 catenin, and α-catenin (Figure 1.2). Through homophilic, Ca^{2+}-dependent interactions, extracellular E-cadherin associates with E-cadherin molecules of adjacent cells (Shapiro & Weis, 2009). β-catenin binds to the catenin-binding domain of intracellular E-cadherin and α-catenin, which associates with the actin cytoskeleton. p120 catenin stabilizes epithelial cell adherens junctions through its interaction with the juxtamembrane domain of E-cadherin molecules (Ishiyama et al., 2010). Cadherin-catenin complexes are rapidly turned over in the absence of p120 catenin, demonstrating a crucial role for p120 catenin in cadherin stability (Davis, Ireton, & Reynolds, 2003). p120 catenin in the cytoplasm modulates the

activities of small Rho family GTPases by inhibiting RhoA and activating Rac1 and Cdc42, which together influence cytoskeletal dynamics and cell migration (Anastasiadis et al., 2000; Noren, Liu, Burridge, & Kreft, 2000).

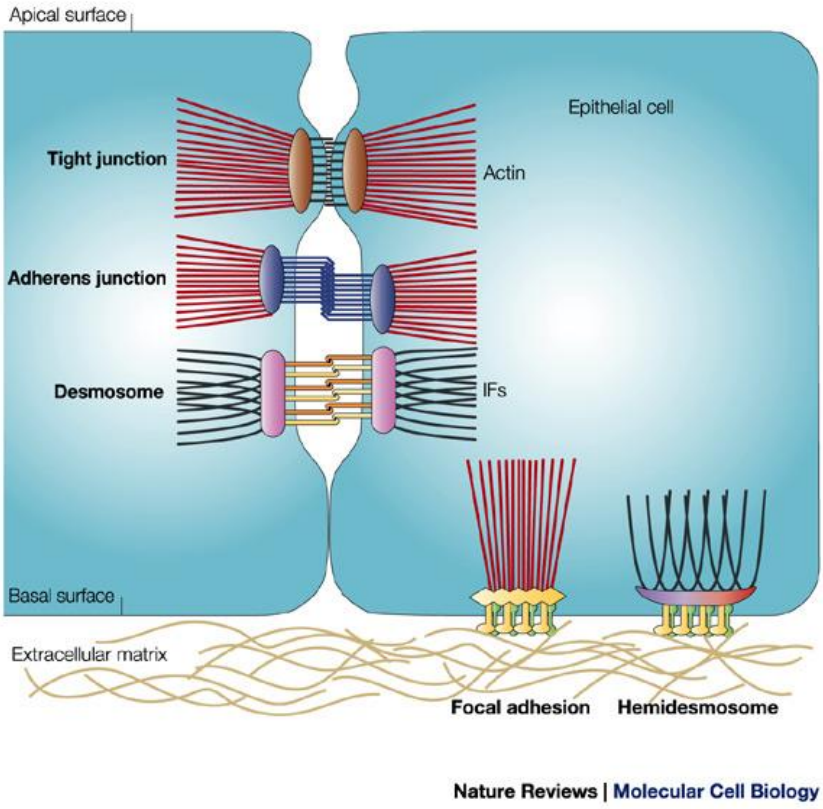

Figure 1.1. Junctional complexes in polarized epithelial cells. Tight junctions are a complex present only in vertebrates; the corresponding complex in invertebrates is septate junctions. Tight junctions or zonula occludens and adherens junctions function to link the actin cytoskeleton of neighboring cells through adapter proteins including claudins and occludens, and cadherins and catenins, respectively. Tight junctions also promote formation of an apical barrier in some polarized epithelial cells. Desmosomes link the intermediate filaments of neighboring cells through proteins which include desmoglein and desmocollin. Focal adhesions and hemidesmosomes connect the cell's actin cytoskeleton and intermediate filaments, respectively, to the extracellular matrix. Image used with permission from Nature Publishing Group.

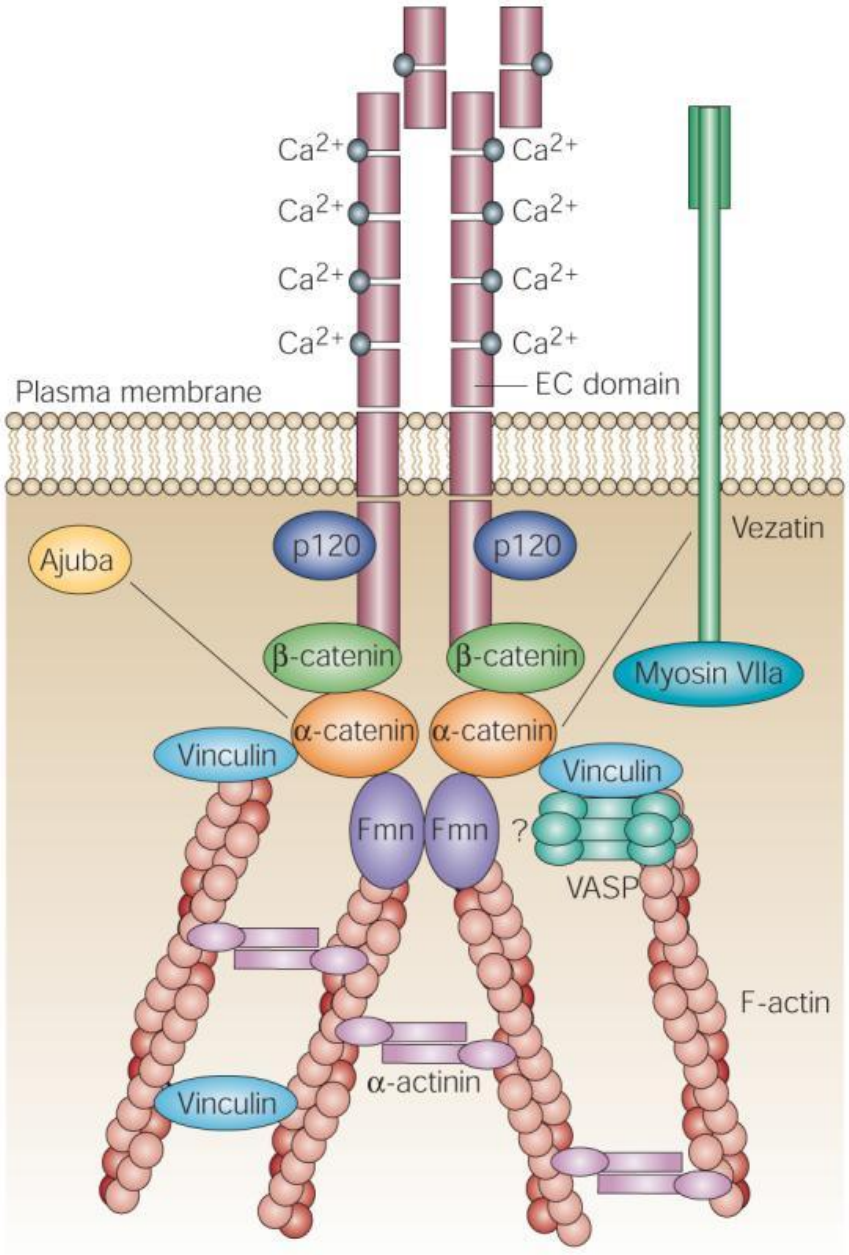

Figure 1.2. Adherens junction complex in epithelial cells. Through calcium dependent interactions, extracellular E-cadherin homodimerizes at cell membranes. The cytoplasmic domain of E-cadherin binds to p120 catenin and β-catenin. β-catenin interacts with α-catenin, which in turn facilitates association with the actin cytoskeleton. Image used with permission from Nature Publishing Group.

Pancreas

The pancreas is a digestive organ comprising two major compartments: the exocrine portion and the endocrine compartment. Pancreatic acinar cells, centroacinar cells and ductal cells make up the exocrine component of the pancreas, while alpha cells, beta cells, delta cells, PP cells and epsilon cells located in the islets of Langerhans constitute the endocrine portion (Figure 1.3). Pancreatic acinar cells synthesize digestive enzymes, including proteases like trypsinogen, chymotrypsinogen, elastase and carboxypeptidase, pancreatic lipase, nucleases and amylase, which are secreted into the extensive branched pancreatic ductal tree and eventually flow into the duodenum to aid in digestion. The pancreatic centroacinar and ductal cells produce a bicarbonate-rich secretion when stimulated by the hormone secretin, which mixes with the acinar cell secretions to compose the pancreatic juice. The bicarbonate secretion functions to neutralize acidic fluids coming from the stomach prior to entry into the duodenum and small intestine.

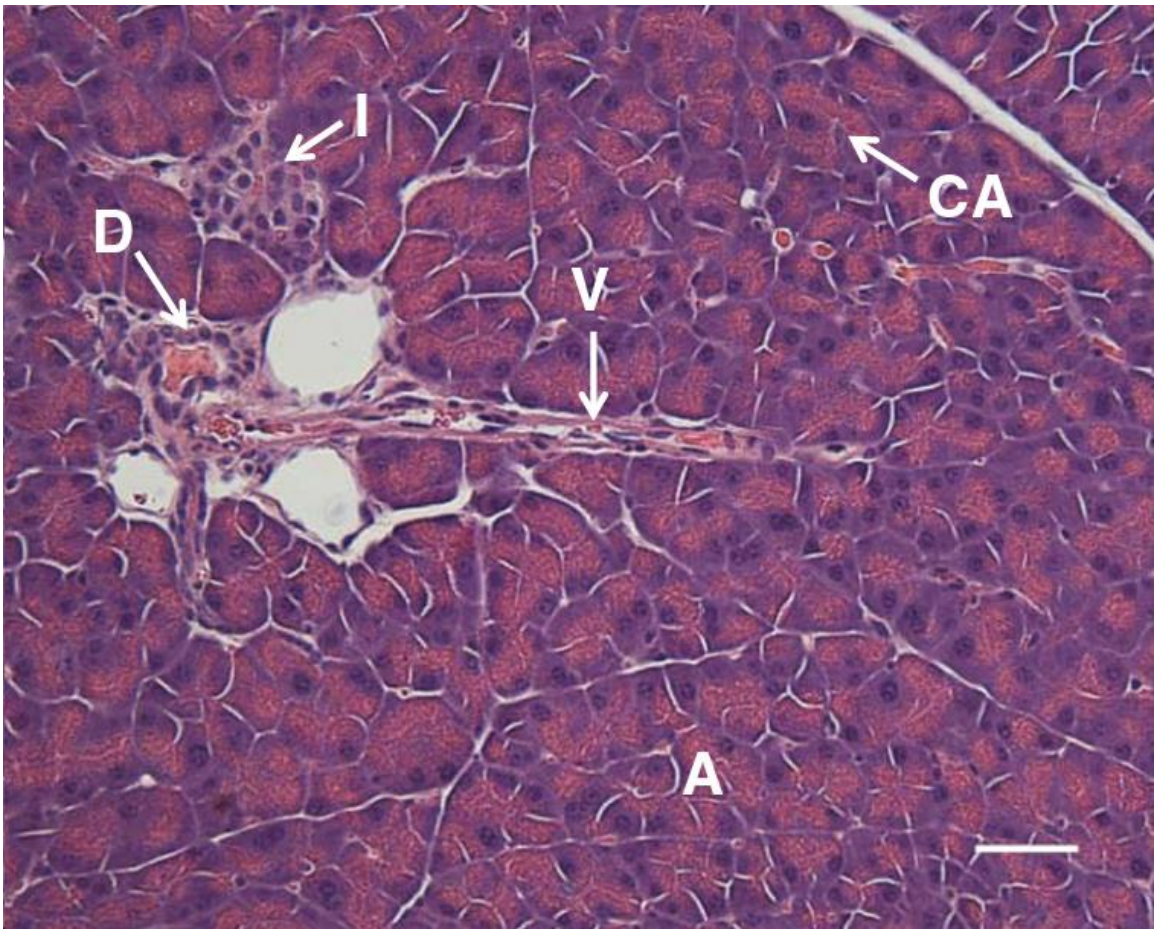

Figure 1.3. Hematoxylin and Eosin staining of the human pancreas. The hormone-producing endocrine gland of the pancreas is composed of the Islets of Langerhans (I). Hormones secreted by islet cells are transported via blood vessels (V). The digestive exocrine compartment is composed of the acinar cells (A), ductal cells (D) and centroacinar cells (CA). Image used with permission from John Wiley and Sons publishing group.

Adherens junctions in mammalian morphogenesis and physiology

Cadherin family molecules play sundry and important roles in mammalian tissue morphogenesis and homeostasis. Cell-cell interactions mediated by E-cadherin during embryonic development are critical for blastula formation in many mammals (Fleming, Papenbrock, Fesenko, Hausen, & Sheth, 2000). N-cadherin, a classic cadherin from the cadherin superfamily and component of adherens junctions, is expressed in many cell types in the body including cardiac muscle and neurons. N-cadherin is required for numerous morphogenetic processes during embryonic mouse development including gastrulation and left-right asymmetry. For pancreas, N-cadherin is expressed in the early murine pancreatic epithelium and surrounding mesenchyme at E9.5, while its expression is restricted to endocrine populations later during pancreas development. Mouse embryos with homozygous loss of N-cadherin in the Pdx-1 endodermal epithelial expression domain, which constitutes early multipotent pancreatic progenitors at E8.5, display selective dorsal pancreatic agenesis (Esni, Johansson, Radice, & Semb, 2001). N-cadherin was also shown to be dispensable for lineage specification during pancreas development and to regulate beta-cell granule turnover in adult mice (J. K. Johansson et al., 2010).

During tissue morphogenesis and maintenance, catenins such as alpha catenin and beta-catenin function in cellular organization and polarity, in part by connecting cadherin molecules to cytoskeletal actin filaments. p120 catenin assists in anchoring cadherin molecules to the cell membrane thereby regulating their stability and turnover (Davis et al., 2003). Dynamic activation of Wnt/beta catenin signaling plays integral roles during mouse embryonic development including body axis patterning and cellular fate

determination, migration, and proliferation, and regulates tissue turnover in a number of adult tissues (Clevers, 2006). Activation of *Ctnnb1* during mouse pancreas development results in neonatal lethality (Heiser, Lau, Taketo, Herrera, & Hebrok, 2006). Conditional deletion of *Ctnnb1* during pancreatic organogenesis resulted in a reduction in endocrine cell numbers, acute edematous pancreatitis in neonates, but maintenance of adherens junctions and desmosomes (Dessimoz, Bonnard, Huelsken, & Grapin-Botton, 2005). Additional studies showed a dramatic reduction of exocrine tissue with no apparent effect on endocrine cells with conditional deletion of *Ctnnb1* during pancreatic organogenesis (Murtaugh, Law, Dor, & Melton, 2005; Wells et al., 2007).

Pancreatic cancer

Pancreatic ductal adenocarcinoma (PDAC) is the most common type of pancreatic cancer, accounting for more than 90% of cases. The majority of patients diagnosed with PDAC have limited options for surgical treatment or chemotherapy. PDAC has the lowest 5-year survival rate of any pancreatic cancer, largely because patients are commonly diagnosed with metastatic disease, as the tumours metastasize early and do not respond well to chemotherapy. For patients for whom surgical intervention is an option, the statistics for survival remain grim, as disease recurrence is common, giving them a 5-year survival rate of about 10-20% (Gopinathan, Morton, Jodrell, & Sansom, 2015). While these properties are common to many types of cancer, for pancreatic cancer they represent an avenue for potential therapeutic intervention. The discovery of a biological mechanism that is primarily responsible for tumour growth and metastases will indelibly aid in the discovery of novel therapies for the treatment of pancreatic cancer.

Adherens junctions in pancreatic tumorigenesis

A sequencing study showed 79% of 24 pancreatic adenocarcinomas have mutations in homophilic cell adhesion molecules including *CDH1* and *CDH2*, the genes encoding E-cadherin and N-cadherin, respectively (Jones et al., 2008). The role of cadherins and catenins have received a lot of attention regarding their contribution to epithelial-mesenchymal transition (EMT), a process that may promote invasion and dissemination of epithelial tumor cells. Downregulation of E-cadherin and β-catenin at cell membranes are hallmarks of EMT (Chaw et al., 2012). Cadherin subtype switching from E-cadherin to N-cadherin is commonly associated with EMT. Conditional ablation of N-cadherin and simultaneous activation of oncogenic Kras in mouse pancreas results in hyperproliferation, accelerated PanIN formation, and early tumor development (Su, Li, Shi, Hruban, & Radice, 2015). Additionally, haploinsufficient expression of N-cadherin in a mouse model of pancreatic cancer results in significantly increased survival (Su et al., 2012).

Mutations in *CTNNB1* have been identified in 100% solid pseudopapillary neoplasms (SPN) of the pancreas (Wu et al., 2011). In mice, activation of *Ctnnb1* alone, which results in up-regulation of Wnt-responsive target genes, is sufficient to drive tumorigenesis from pancreatic epithelium and promotes formation of tumors which histologically resemble SPN. Interestingly, in this model stabilization of β-catenin in cooperation with oncogenic Kras also prevented formation of PanIN (Heiser et al., 2008). Additional mouse modeling showed that ligand-mediated activation of the Wnt/β-catenin pathway is required to initiate pancreatic cancer (Zhang et al., 2013).

Aims of Thesis

Interest in adherens junction molecules has precipitated in light of discovery that their biological activities include essential roles in mammalian development, normal tissue physiology, and the ability to contribute to tumorigenesis when these normal functions are perturbed. The *in vivo* pancreatic function of a key regulator of E-cadherin stability and turnover, p120 catenin, has not previously been studied. The aims of this thesis are to elucidate the role of p120 catenin in pancreas development, normal physiology, and tumorigenesis in an effort to lend insight into its therapeutic potential. An investigation into the expression of p120 catenin revealed ubiquitous expression in normal human pancreas and downregulation in human pancreatic adenocarcinoma and metastasis, observations which warrant further studies to determine the mechanisms by which p120 catenin potentially contributes to each of these processes. While various biological activities can be studied *in vitro*, a more complete understanding of the functional role of p120 catenin requires animal models. The development of transgenic mice with both spatially and temporally controlled expression of p120 catenin provided the necessary tool to assess the developmental, physiological, and tumorigenic capacity of p120 catenin. This approach has the added benefit of enabling examination of biologically relevant pathways as well as the ability to examine potential effects on multiple organ systems in the context of a primary pancreatic insult. Given the established role for adherens junctions in disease pathology, future studies which incorporate both functional elucidation as well as disease mechanisms have the greatest potential for yielding therapeutic targets.

CHAPTER 2 - P120 CATENIN IS REQUIRED FOR NORMAL TUBULOGENESIS BUT NOT EPITHELIAL INTEGRITY IN DEVELOPING MOUSE PANCREAS

INTRODUCTION

Pancreatic development proceeds from a cluster of endodermal epithelial cells that give rise to a highly specialized, heterogeneous endocrine and exocrine organ. The early pancreatic bud is enveloped by mesenchyme, which is required for pancreas development and is thought to provide inductive signals for the specification of various cell types (Golosow & Grobstein, 1962; Landsman et al., 2011). Beginning at E13.5, the mouse pancreas changes rapidly during the 'secondary transition,' which is marked by dramatic increases in endocrine cell numbers and acinar cell differentiation (Rutter et al., 1968). Endocrine cells delaminate from the embryonic epithelia, coalesce into early islets, and migrate throughout the tissue (Pictet, Clark, Williams, & Rutter, 1972). The developing pancreas arborizes to generate a highly branched network of exocrine tissue consisting of acini capping the tips of terminal ducts and extending to the main pancreatic duct (Puri & Hebrok, 2007; Villasenor, Chong, Henkemeyer, & Cleaver, 2010).

p120 catenin is a member of the larger catenin gene family that is comprised of three subfamilies; p120, beta, and alpha. The p120 subfamily contains 7 members which include p120 catenin, ARVCF, δ-catenin, p0071, and plakophilins 1-3 (Zhao, Reynolds, & Gaucher, 2011). Like p120 catenin, ARVCF, δ-catenin, and p0071 are capable of binding to the juxtamembrane domain of cadherin molecules in adherens junctions through their central Armadillo repeat domains, while plakophilins 1-3 primarily function in linking the intermediate filaments of cells through desmosomes. p120 catenin,

ARVCF, and p0071 are expressed ubiquitously and as multiple isoforms. Expression of δ-catenin is thought to be restricted almost entirely to the nervous system (Mariner, Wang, & Reynolds, 2000; Pieters, van Hengel, & van Roy, 2012). The biological interplay between p120 catenin and its family members is incompletely understood, especially during development.

Deletion of p120 catenin by conventional homologous recombination in mice results in embryonic lethality (Davis & Reynolds, 2006). Therefore, a number of studies using targeted conditional gene ablation strategies in different murine organ systems have revealed various *in vivo* roles for p120 catenin. In mouse salivary gland organogenesis, p120 catenin ablation results in blocked acinar cell differentiation, E-cadherin and β-catenin reduction, early post-natal death with occlusion of abnormally expanded ducts, and progression to a pre-cancer intraepithelial neoplasia phenotype (Davis and Reynolds, 2006). Deletion of p120 catenin in the epidermis results in cell autonomous activation of nuclear factor kappa B (NFkB) signaling, providing evidence to link p120 catenin to inflammation (Perez-Moreno et al., 2006). Mice with p120 catenin-null endothelium exhibit defects in vascular morphogenesis and angiogenic remodeling, accompanied by hemorrhage, and midgestational embryonic lethality (Oas et al., 2010). Studies have also revealed critical yet diverse roles for p120 catenin in dental enamel, renal, dendrite, mammary gland, and eye development (Bartlett et al., 2010; Elia et al., 2006; Kurley et al., 2012; Marciano et al., 2011; Tian et al., 2012). Conditional ablation of p120 catenin has also revealed crucial roles in organ homeostasis and tumorigenesis (Perez-Moreno et al., 2008; Schackmann et al., 2013; Smalley-Freed et al., 2010; Smalley-Freed et al.,

2011; Stairs et al., 2011). In these studies, the results of p120 catenin deletion are highly tissue-specific and unpredictable.

RESULTS

Characterization of p120 catenin expression in mouse pancreas

We first sought to determine baseline expression of p120 catenin in the embryonic, neonatal, and adult mouse pancreas. To accomplish this, we used immunofluorescence (IF) with an antibody that recognizes isoforms 1-4 of p120 catenin and also an antibody specific for isoforms 1-3 of p120 catenin. Immunolabeling with an antibody that recognizes isoforms 1-4 of p120 catenin, E-cadherin, and Vimentin showed ubiquitous expression of p120 catenin in both the pancreatic epithelium and the pancreatic mesenchyme throughout development, and in the neonatal and adult pancreatic epithelium (Figure 2.1). At E12.5 and E14.5, p120 catenin labeling was more highly enriched in pancreatic epithelium over pancreatic mesenchyme, except in the endocrine clusters at E12.5, which displayed low levels of adherens junction proteins E-cadherin and p120 catenin. Immunostaining using an antibody specific for isoforms 1-3 of p120 catenin showed a labeling pattern indistinguishable from the isoforms 1-4 specific antibody (Figure 2.2). A schematic of pancreas development is depicted in Figure 2.3.

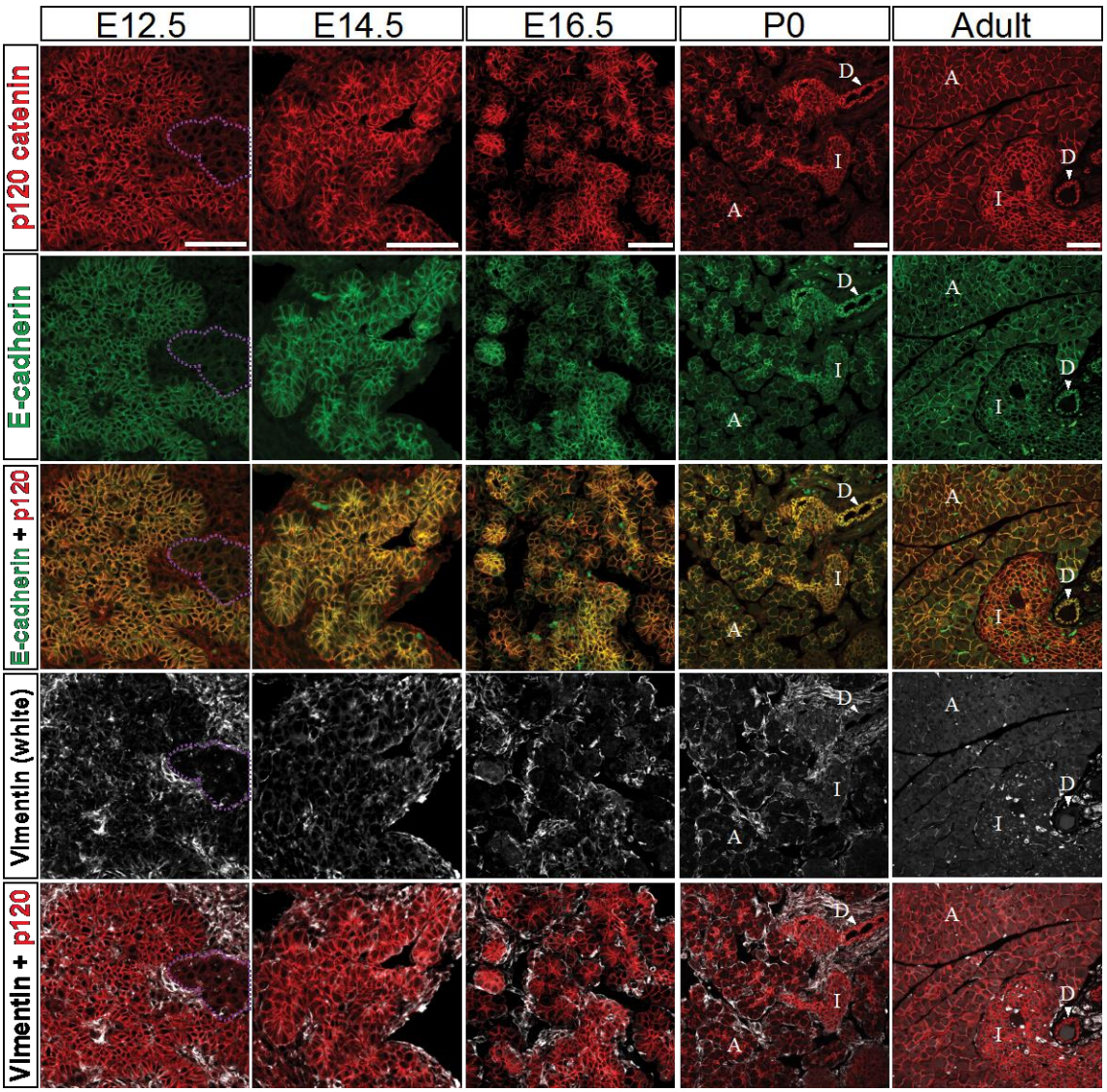

Figure 2.1. p120 catenin is expressed ubiquitously in the embryonic, neonatal, and adult mouse pancreas. Immunostaining showing p120 catenin expression using an antibody specific for isoforms 1-4, E-cadherin, and Vimentin in developing, neonatal, and adult mouse pancreas. For E12.5, purple dotted lines show examples of early endocrine clusters. For neonatal and adult pancreas, white arrows point to cells indicated with a "D" for ducts, "I" indicates islets of Langerhans, and "A" points out acinar cells. Scale bars are 50µm.

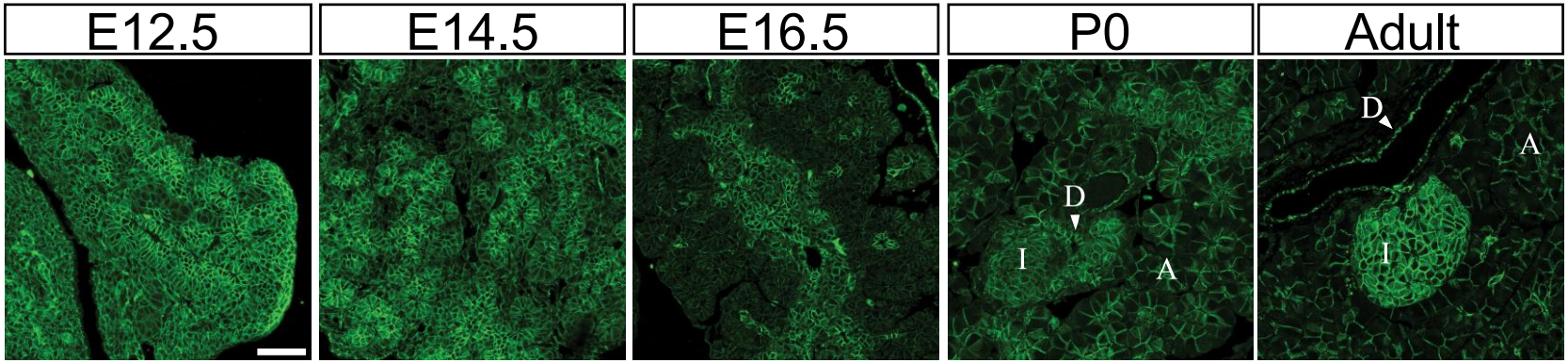

Figure 2.2. p120 catenin expression of isoforms 1-3. Immunolabeling using an antibody specific for isoforms 1-3 of p120 catenin showed ubiquitous expression of p120 catenin in developing, neonatal, and adult mouse pancreas. For neonatal and adult pancreas, white arrows show cells indicated with a "D" for ducts, "I" indicates islets of Langerhans, and "A" designates acinar cells. Scale bar is 50μm.

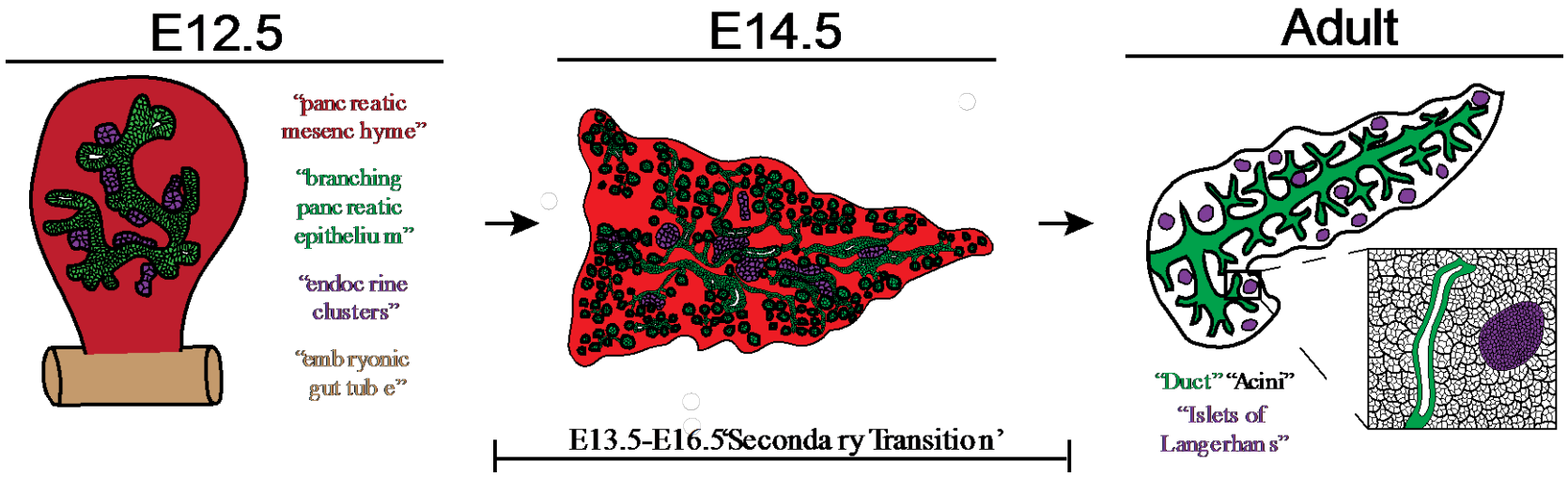

Figure 2.3. Illustration of pancreas development. Schematic depiction of branching pancreatic epithelium at E12.5 with emerging endocrine clusters (left), arborization of the pancreatic epithelium at E14.5 with accompanying endocrine cluster migration (middle), and an illustration of the adult pancreas containing duct, islet, and endocrine cells (right). For E12.5 and E14.5, various cell types of the developing pancreas are color coded, with red indicating mesenchymal cells, green indicating tubular epithelium, and purple indicating endocrine clusters. The time frame for the 'secondary transition' during pancreatic development is also depicted. For the adult illustration, green represents ducts, purple represents islets of Langerhans, and black indicates differentiated acinar cells.

Loss of p120 catenin during development disrupts pancreatic tissue architecture

$C^{Pdx1}; p120^{f/wt}$ mice were bred with $p120^{f/f}$ mice, irrespective of gender, to generate $C^{Pdx1}; p120^{f/f}$ homozygotes. $p120^{f/f}$ mice harbor floxed p120 catenin alleles with loxP sites flanking all four known translational start sites; thus, genetic recombination should prevent expression of all isoforms of p120 catenin (Davis & Reynolds, 2006). Offspring from crosses were born in Mendelian ratios, but 100% of female $C^{Pdx1}; p120^{f/f}$ homozygotes died during the early post-natal period while male $C^{Pdx1}; p120^{f/f}$ homozygotes survived to adulthood.

Pancreatic histology of $C^{Pdx1}; p120^{f/f}$ and wild-type littermate controls was initially examined at P0, revealing profound changes in pancreatic epithelial architecture in homozygous $p120^{f/f}$ pancreata (Figure 2.4A). $C^{Pdx1}; p120^{f/f}$ pancreata displayed a dramatic expansion of tubular epithelium, which was positive for the epithelial marker Cytokeratin 19 (Figure 2.4A,B). A substantial number of Vimentin$^+$ cells were centrally located in the pancreata of homozygous $p120^{f/f}$ animals (Figure 2.5). Amylase IF demonstrated an overall defect in mature acinar cell differentiation and distribution in homozygous $p120^{f/f}$ pancreata when compared to controls (Figure 2.4C). Acinar units in homozygous $p120^{f/f}$ animals were peripherally located in early pancreatic lobules and often displayed expanded lumens. Transitional structures with both acinar and duct morphologies were also evident (Figure 2.4A,B). The abnormal epithelial tubules and transitional structures morphologically resembled areas of ADM, which occur in the human pancreas in the setting of either pancreatitis or early pancreatic cancer.

IF demonstrated epithelial-specific loss of p120 catenin in homozygous $p120^{f/f}$ pancreata (Figure 2.4D). As expected, expression of p120 catenin in the pancreatic

mesenchyme was unaffected (Figure 2.4D). We observed a small degree of mosaicism for recombination of the floxed p120 catenin allele during development. Epithelium retaining p120 catenin accounted for 6.07% ± 2.49% pancreatic cells during development (n=7) and 59.77% ± 3.31% pancreatic cells in $C^{Pdx1}; p120^{f/f}$ adult males (n=6). This suggests that there may be a selection bias for cells that retain expression of p120 catenin. Islets were abnormally distributed throughout the tissue in a pattern such that they surrounded the central Vimentin$^+$ cells in homozygous $p120^{f/f}$ pancreata. The differentiation of α and β cells, marked by Glucagon and C-peptide, respectively, was unaffected throughout embryonic development and in the neonatal pancreas (Figure 2.4E and data not shown). No difference was observed in the number of proliferating cells or cells undergoing apoptosis in the pancreatic epithelial or mesenchymal compartments when comparing wild-type and homozygous $p120^{f/f}$ animals, as examined by cleaved Caspase-3 and Ki67 IF (Figure 2.6, 2.7). $C^{Pdx1}; p120^{f/wt}$ pancreata were histologically indistinguishable from wild-type pancreata both during development and in adulthood, suggesting that a single wild-type allele of p120 catenin is sufficient for normal pancreatic development and homeostasis (data not shown).

Loss of p120 catenin causes inflammation in the neonatal and adult pancreas

The overt phenotype observed at P0 resembled pancreatitis, with regions containing abundant Vimentin$^+$ cells (Figure 2.5), expanded epithelial tubules, and ADM-like transitional structures. Therefore, we next hypothesized that p120 catenin loss might induce inflammation in the neonatal pancreas. To test this hypothesis, we stained for CD45 in $C^{Pdx1}; p120^{f/f}$ and wild-type control pancreata. Abundant CD45$^+$ cells were

detected in $C^{Pdx1}; p120^{f/f}$ P0 pancreata but not wild-type controls, indicating the recruitment of an inflammatory infiltrate in $C^{Pdx1}; p120^{f/f}$ pancreata (Figure 2.4F). In an effort to understand the mechanism of induction of inflammation in neonatal pancreas, we next stained for a known regulator of inflammation that has been previously associated with p120 catenin loss and states of pancreatic inflammation, NF-kB (Perez-Moreno et al., 2006; Rakonczay, Hegyi, Takacs, McCarroll, & Saluja, 2008; Stairs et al., 2011). NF-kB was detected in ductal epithelium in wild-type pancreata as well as in expanded ductal epithelium in $C^{Pdx1}; p120^{f/f}$ pancreata at P0 (Figure 2.4G). As incorrect regulation of NF-kB has been linked to dysregulation of the immune system and inflammation (Perez-Moreno et al., 2006; Stairs et al., 2011), widespread pancreatic upregulation of NF-kB in expanded ductal epithelium of $C^{Pdx1}; p120^{f/f}$ pancreata may represent a possible mechanism for the induction of inflammation.

We next sought to determine if p120 catenin also regulated inflammation in adult pancreas. We examined the pancreatic histology of cohorts of animals ranging in age from 1 to 12 months. At 1 month of age, 2/4 $C^{Pdx1}; p120^{f/f}$ males displayed frank pancreatitis (Figure 2.8). Over time, these animals also developed dilated main pancreatic ducts, chronic pancreatitis, ADM, and mucinous metaplasia resembling PanIN1a, with incomplete penetrance (4 out of 7 mice) (Figure 2.8). Remaining adult homozygous $p120^{f/f}$ pancreata were histologically normal. Adult $C^{Pdx1}; p120^{f/f}$ animals also displayed abundant $CD45^{+}$ cells in regions characterized by pancreatitis (Figure 2.8). Incomplete penetrance of the phenotype in adult pancreata might be influenced by the degree of mosaicism for loss of p120 catenin. Immunolabeling for p120 catenin revealed that ADM

and mucinous metaplastic lesions lack p120 catenin, suggesting that these lesions may represent cell autonomous sequelae of p120 catenin deletion (Figure 2.8).

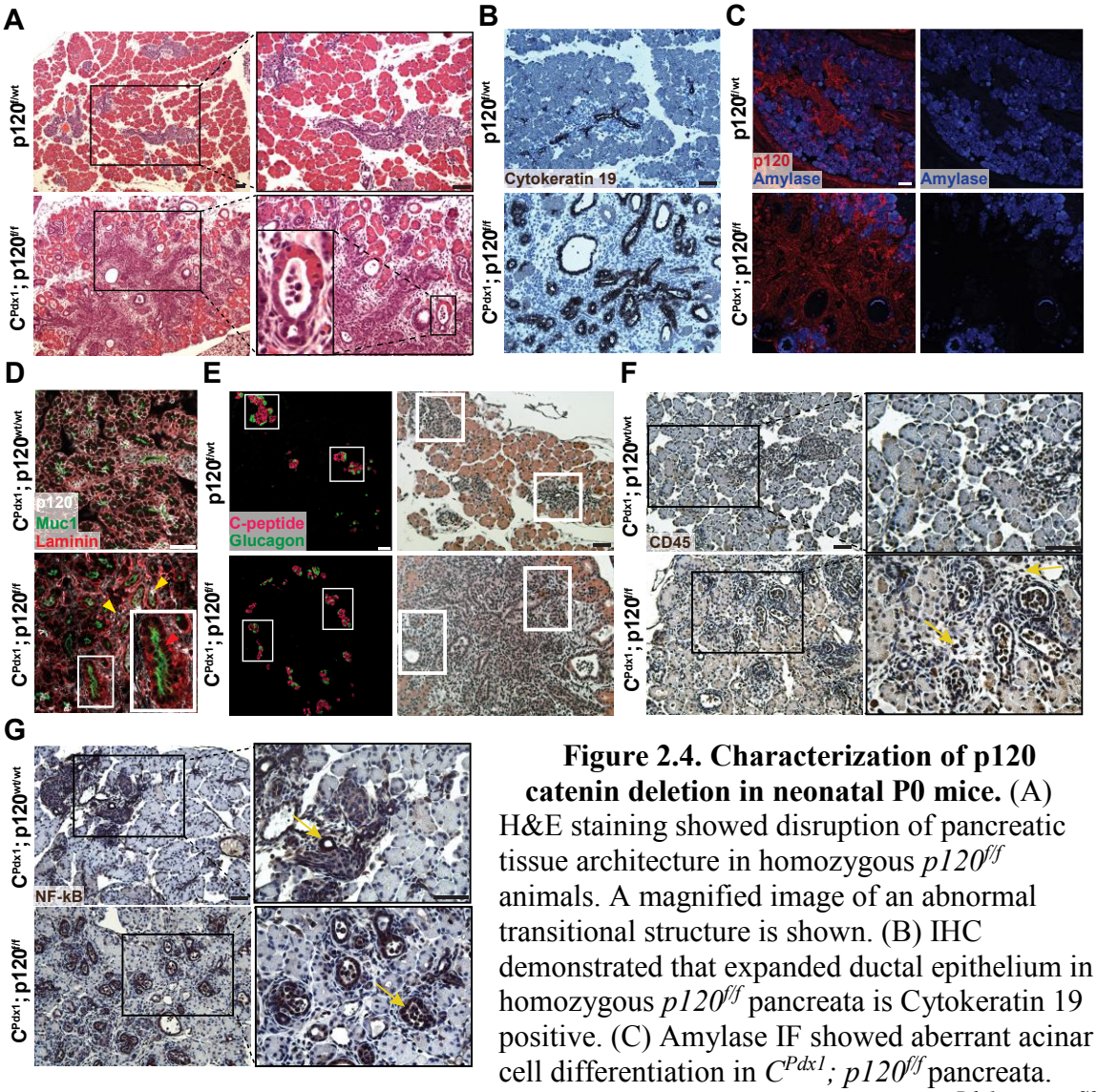

Figure 2.4. Characterization of p120 catenin deletion in neonatal P0 mice. (A) H&E staining showed disruption of pancreatic tissue architecture in homozygous *p120$^{f/f}$* animals. A magnified image of an abnormal transitional structure is shown. (B) IHC demonstrated that expanded ductal epithelium in homozygous *p120$^{f/f}$* pancreata is Cytokeratin 19 positive. (C) Amylase IF showed aberrant acinar cell differentiation in *C^{Pdx1}; p120$^{f/f}$* pancreata. (D) IF imaging documented epithelial-specific p120 catenin deletion. In the *C^{Pdx1}; p120$^{f/f}$* figure, the top right yellow arrow points to a rare p120 catenin-expressing epithelial cell. The left yellow arrow points to a p120 catenin-expressing non-epithelial cell. The red arrow in the inset shows epithelia in *C^{Pdx1}; p120$^{f/f}$* pancreas that lack p120 catenin. Note that p120 catenin staining is nearly completely absent in *C^{Pdx1}; p120$^{f/f}$* epithelial pancreas, with very few cells mosaic for p120 catenin expression. Muc1 and Laminin provide clear boundaries for distinction of apical and basal regions of the pancreatic epithelium. (E) IF of C-peptide and Glucagon demonstrated abnormal islet distribution in homozygous *p120$^{f/f}$* pancreas. Identical sections were stained with H&E to demonstrate tissue context, and white boxes show corresponding islets. (F) CD45 IHC revealed the recruitment of an inflammatory infiltrate in *C^{Pdx1}; p120$^{f/f}$* pancreas, but not in wild-type controls. In the *C^{Pdx1}; p120$^{f/f}$* bottom panel, yellow arrows point to CD45^{+} cells. (G) NF-kB signaling was detected in the ductal epithelium of both wild-type and homozygous *p120$^{f/f}$* pancreas, which is indicated by yellow arrows. Scale bars are 50µm.

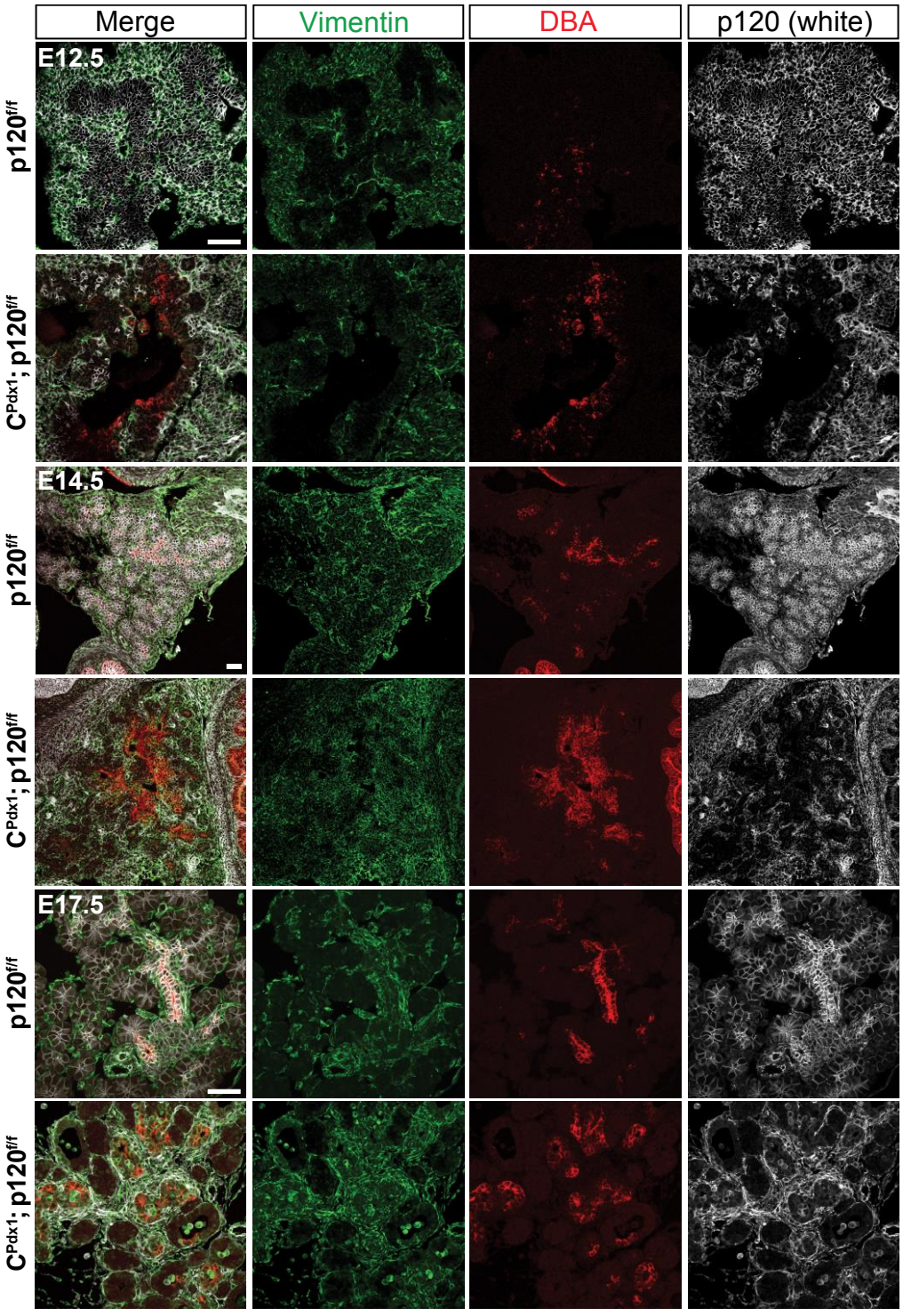

Figure 2.5. Homozygous *p120$^{f/f}$* pancreata display increased Vimentin^{+} cells and duct-like epithelium during development. Expansion of a centralized Vimentin^{+} cluster of cells and DBA^{+} epithelium in homozygous *p120$^{f/f}$* pancreas was evident as early as E14.5. Some expanded epithelial tubules lost expression of the ductal marker DBA at E17.5, but retained expression of Cytokeratin 19 at P0 (Fig. 2.4B). Scale bars are 50µm.

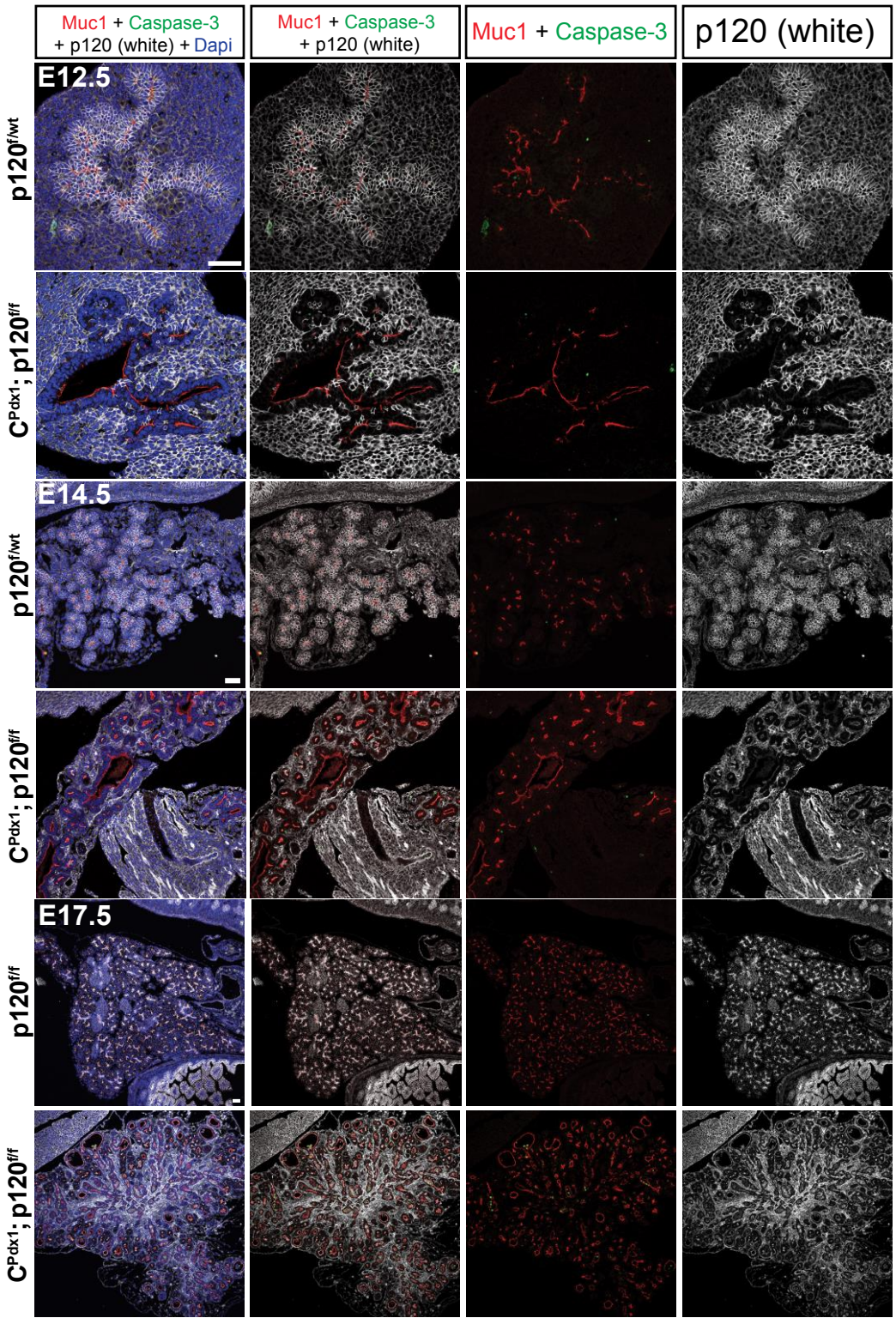

Figure 2.6. Apoptosis is comparable in *C^{Pdx1}; p120$^{f/f}$* and littermate control pancreata throughout development. (A-C) No difference was detected in Cleaved Caspase-3 staining throughout development in *C^{Pdx1}; p120$^{f/f}$* and littermate control pancreata. Scale bars are 50µm.

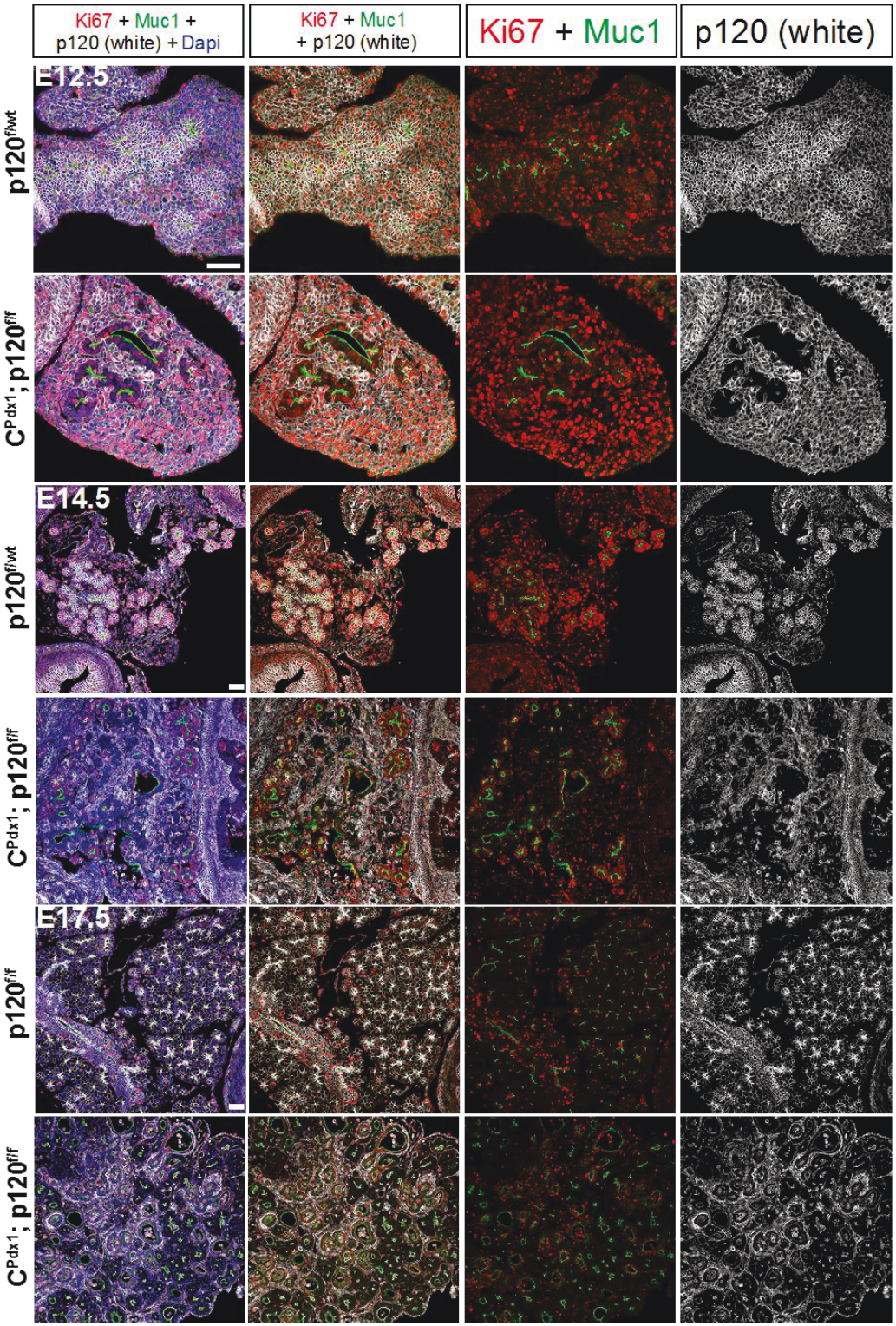

Figure 2.7. Proliferation is comparable in *C^{Pdx1}; p120$^{f/f}$* and littermate control pancreata throughout development. No difference was detected in Ki67 staining throughout development in *C^{Pdx1}; p120$^{f/f}$* and littermate control pancreata. Scale bars are 50µm.

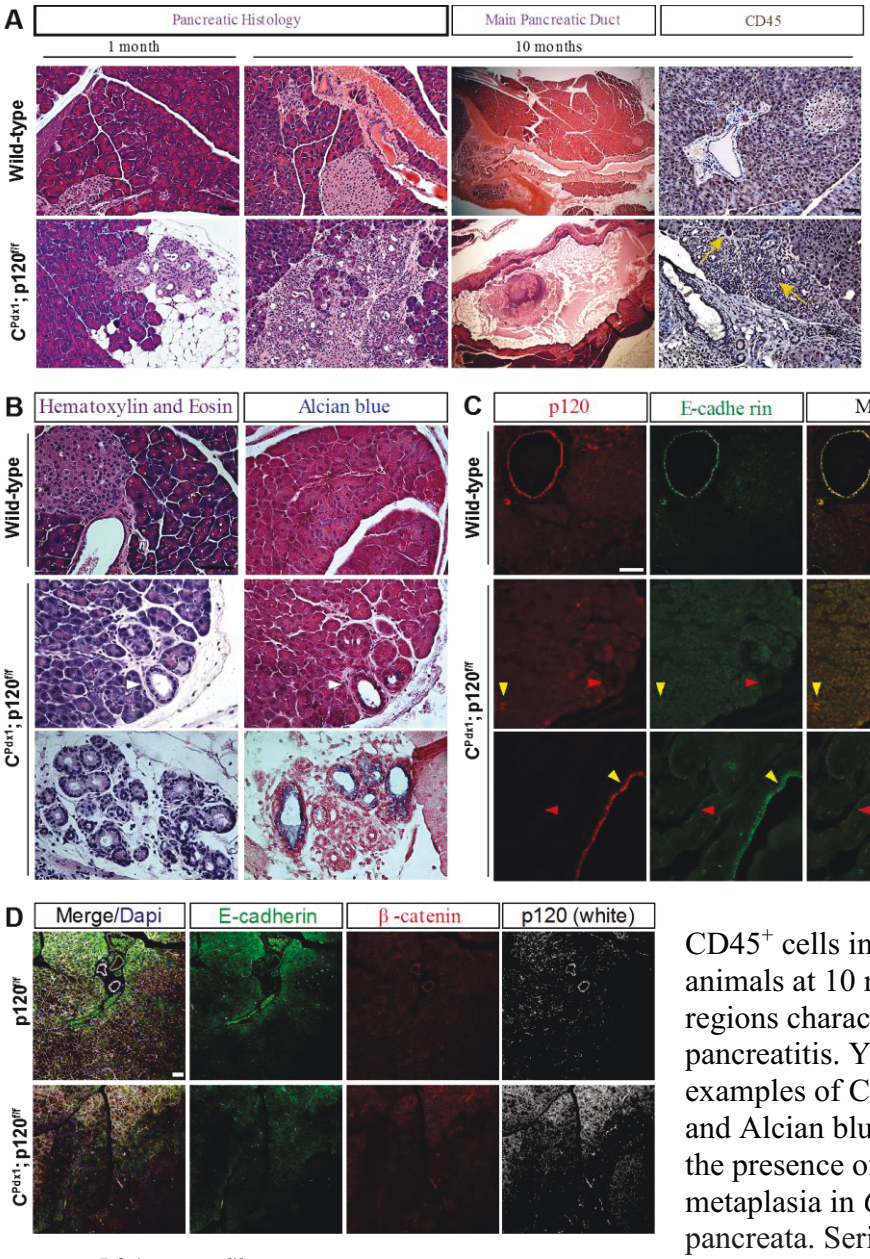

Figure 2.8. Adult *C^{Pdx1}; p120$^{f/f}$* phenotype. (A-B) Adult *C^{Pdx1}; p120$^{f/f}$* males displayed chronic pancreatitis, dilated main pancreatic ducts, ADM, and mucinous metaplasia that resembled PanIN1a. (A) Pancreatitis and fatty replacement of acinar tissue was evident in *C^{Pdx1}; p120$^{f/f}$* animals as early as 1 month. IHC showed CD45^{+} cells in *C^{Pdx1}; p120$^{f/f}$* animals at 10 months of age in regions characterized by pancreatitis. Yellow arrows point to examples of CD45^{+} cells. (B) H&E and Alcian blue staining revealed the presence of mucinous metaplasia in *C^{Pdx1}; p120$^{f/f}$* pancreata. Serial sections were used in the *C^{Pdx1}; p120$^{f/f}$* panel for H&E and Alcian blue staining. (C) E-cadherin and p120 catenin immunostaining in animals 10-12 months of age demonstrated that ADM lesions lack p120 catenin in *C^{Pdx1}; p120$^{f/f}$* pancreata, suggesting that these lesions may be forming in a cell autonomous manner. The bottom panel of images depicts mosaicism for p120 catenin expression in the main pancreatic duct of *C^{Pdx1}; p120$^{f/f}$* animals. Yellow arrows point to p120 catenin-expressing cells while red arrows show p120 catenin-null cells. (D) IF demonstrated mosaicism for p120 catenin, E-cadherin, and β-catenin in adult *C^{Pdx1}; p120$^{f/f}$* pancreata 10 months of age. Note that (C) and (D) collectively demonstrate that unlike embryonic pancreas, adult pancreatic epithelium lacking p120 catenin does not retain adherens junction proteins E-cadherin and β-catenin. Scale bars are 50μm.

p120 catenin is required for normal pancreatic tubulogenesis and branching morphogenesis

To examine the p120 catenin loss-of-function phenotype during development, we next undertook a histological examination of wild-type and homozygous $p120^{f/f}$ pancreata in a developmental series from E12.5 through E17.5. $C^{Pdx1}; p120^{f/f}$ pancreata displayed decreased ramification of branches and expanded epithelial tubule lumen diameter throughout development (Figure 2.9A). Aberrant tubulogenesis became evident as early as E12.5 and epithelial tubules continued to show expanded lumens through E17.5. An increased proportion of duct-like epithelium became evident at E14.5 (Figure 2.5) and was found distributed throughout the tissue by E16.5 (Figure 2.9A). Proper acinar cell differentiation was impeded throughout development in homozygous $p120^{f/f}$ animals.

As we identified that homozygous deletion of p120 catenin resulted in the recruitment of an inflammatory environment during pancreatogenesis, we next asked if the aberrant tubulogenesis evident at E12.5 would still occur in the absence of an inflammatory infiltrate. To address this question, we employed a double fluorescent mTmG reporter allele (hereafter denoted as "mTmG") to visualize the dynamics of pancreatic tubulogenesis in dissected pancreatic anlagen in an inflammation-free *in vitro* setting. $C^{Pdx1}; p120^{f/wt}; mTmG^{Tg/Tg}$ mice were bred with $p120^{f/wt}$ mice to generate $C^{Pdx1}; p120^{wt/wt}; mTmG^{Tg/wt}$, $C^{Pdx1}; p120^{f/wt}; mTmG^{Tg/wt}$, and $C^{Pdx1}; p120^{f/f}; mTmG^{Tg/wt}$ mice. Pancreatic rudiments including surrounding mesenchyme, caudal stomach, and proximal duodenum were dissected at E11.5 and cultured for up to 7 days as previously described (Puri & Hebrok, 2007). Aberrant pancreatic tubulogenesis similar to that observed *in vivo* was also found to occur *in vitro* in $C^{Pdx1}; p120^{f/f}; mTmG^{Tg/wt}$ mice and not in wild-type

controls. This suggests that the aberrant pancreatic tubulogenesis observed in homozygous $p120^{f/f}$ mice occurred even in the absence of concomitant inflammation (Figure 2.9B). As expected given their normal histological phenotype *in vivo*, pancreatic explants from $C^{Pdx1}; p120^{f/wt}; mTmG^{Tg/wt}$ mice recapitulated normal pancreatic tubulogenesis *in vitro* (data not shown).

Pancreatic progenitor markers are retained in expanded tubular epithelium

Because we identified an alteration in the relative abundance of specific pancreatic cell types in $C^{Pdx1}; p120^{f/f}$ animals, we next evaluated expression of known progenitor markers in developing pancreatic epithelia as a means to assess progenitor differentiation. We examined the pancreatic progenitor markers Sox9, Pdx1, and Aldh1 in a developmental series using IF and immunohistochemistry (IHC). Pdx1 and Sox9 were expressed in the vast majority of pancreatic epithelial cells at E12.5. During development, Pdx1 expression gradually became restricted to endocrine cells, while Sox9 expression became restricted to duct-like epithelium and centroacinar cells (Figure 2.10A). Pdx1 localization in homozygous $p120^{f/f}$ pancreata is unaltered in the developing pancreas when compared to wild-type controls (Fig 2.10A). Between E12.5 and E14.5, Aldh1 was expressed in the tips of normal branching pancreatic epithelia (Figure 2.10B) (Rovira et al., 2010). By P0, wild-type Aldh1 expression was restricted to endocrine cells, a subset of ductal cells, and centroacinar cells (Figure 2.10B). In the absence of p120 catenin, Aldh1 was expressed in the expanded tubular epithelium throughout development, as was Sox9 (Figure 2.10A, 2.10B). Since the early pancreatic progenitor marker Aldh1 is expressed in a subset of ductal cells at P0 in wild-type pancreata,

continued widespread expression of Aldh1 in expanded duct-like epithelium of homozygous *p120$^{f/f}$* pancreata might represent a more underdeveloped, undifferentiated cellular state suggestive of disrupted progenitor differentiation, as has been previously reported for forced ongoing expression of the early pancreatic progenitor marker Sox9 (Seymour et al., 2007).

Loss of p120 catenin alters cytoskeletal organization but not cell polarity

As an additional means to assess the p120 catenin loss-of-function phenotype, we next undertook an examination of apical and basal markers in *C^{Pdx1}; p120$^{f/f}$* and control pancreata. MUC1 is a transmembrane glycoprotein with extensive O-glycosylation that is localized to the apical surface of pancreatic epithelial cells (Hollingsworth & Swanson, 2004; X. Liu, Yi, et al., 2014). We examined localization of the apical marker MUC1 at P0 in *C^{Pdx1}; p120$^{f/f}$* and littermate control pancreata by IF and found that MUC1 was localized apically in both homozygous *p120$^{f/f}$* animals and wild-type controls in both pancreatic ducts and acinar cells (Figure 2.11, 2.12). Phalloidin binds specifically to f-actin filaments, and Phalloidin has been shown to be localized apically and laterally in pancreatic acinar cells (O'Konski & Pandol, 1990; Wulf, Deboben, Bautz, Faulstich, & Wieland, 1979). We found that Phalloidin localization was apical, lateral, and basal in ductal epithelia, and apical in the acini of *C^{Pdx1}; p120$^{f/f}$* pancreata at P0, which differs from the apical localization of Phalloidin in ducts of wild-type pancreata (Figure 2.11).

In an effort to understand atypical actin cytoskeleton organization in expanded ductal epithelium of *C^{Pdx1}; p120$^{f/f}$* pancreata, we next assessed localization of PKCζ, a known cytoplasmic regulator of actin cytoskeleton dynamics (Even-Faitelson & Ravid, 2006; Gomez et al., 1995; Guo et al., 2009; X. J. Liu et al., 2007; Uberall et al., 1999).

PKCζ/Par6/Par3, a complex activated by Cdc42, is associated with the assembly of tight junctions, apical polarization of epithelial cells, and microtubule organization (Baluch & Capco, 2008; Etienne-Manneville & Hall, 2001; Izumi et al., 1998; A. Johansson, Driessens, & Aspenstrom, 2000; Lin et al., 2000; Qiu, Abo, & Steven Martin, 2000). PKCζ/Par6/Par3, when associated with tight junctions is located at the apical surface of epithelial cells (Ebnet et al., 2001). Since p120 catenin activates Cdc42, and Cdc42 activates PKCζ, we next hypothesized that assembly of the PKCζ/Par3/Par6 complex associated with tight junctions might be impaired in the expanded ductal epithelium of homozygous $p120^{f/f}$ pancreata. To address this, we immunostained for PKCζ, which revealed an increase in cytoplasmic PKCζ in expanded ductal epithelium of $C^{Pdx1}; p120^{f/f}$ pancreata when compared to controls (Figure 2.13). Therefore, these results suggest a connection between increased cytoplasmic availability of the cytoskeletal remodeler PKCζ and the observed defects in actin cytoskeletal rearrangement associated with p120 catenin loss.

We next assessed basal polarity by examination of Integrin alpha 6/CD49f using IF. At P0, both $C^{Pdx1}; p120^{f/f}$ and littermate control pancreata had basal localization of CD49f in ductal and acinar epithelia (Figure 2.11). As another assessment of basal polarity, we immunostained for Laminin. Both $C^{Pdx1}; p120^{f/f}$ and littermate control pancreata had basal extracellular localization of Laminin (Figure 2.12). Taken together, these data suggest that basal polarity is maintained, but actin cytoskeleton organization is not completely intact in expanded ductal epithelium of homozygous $p120^{f/f}$ pancreata, an observation which might be mediated by increased cytoplasmic PKCζ.

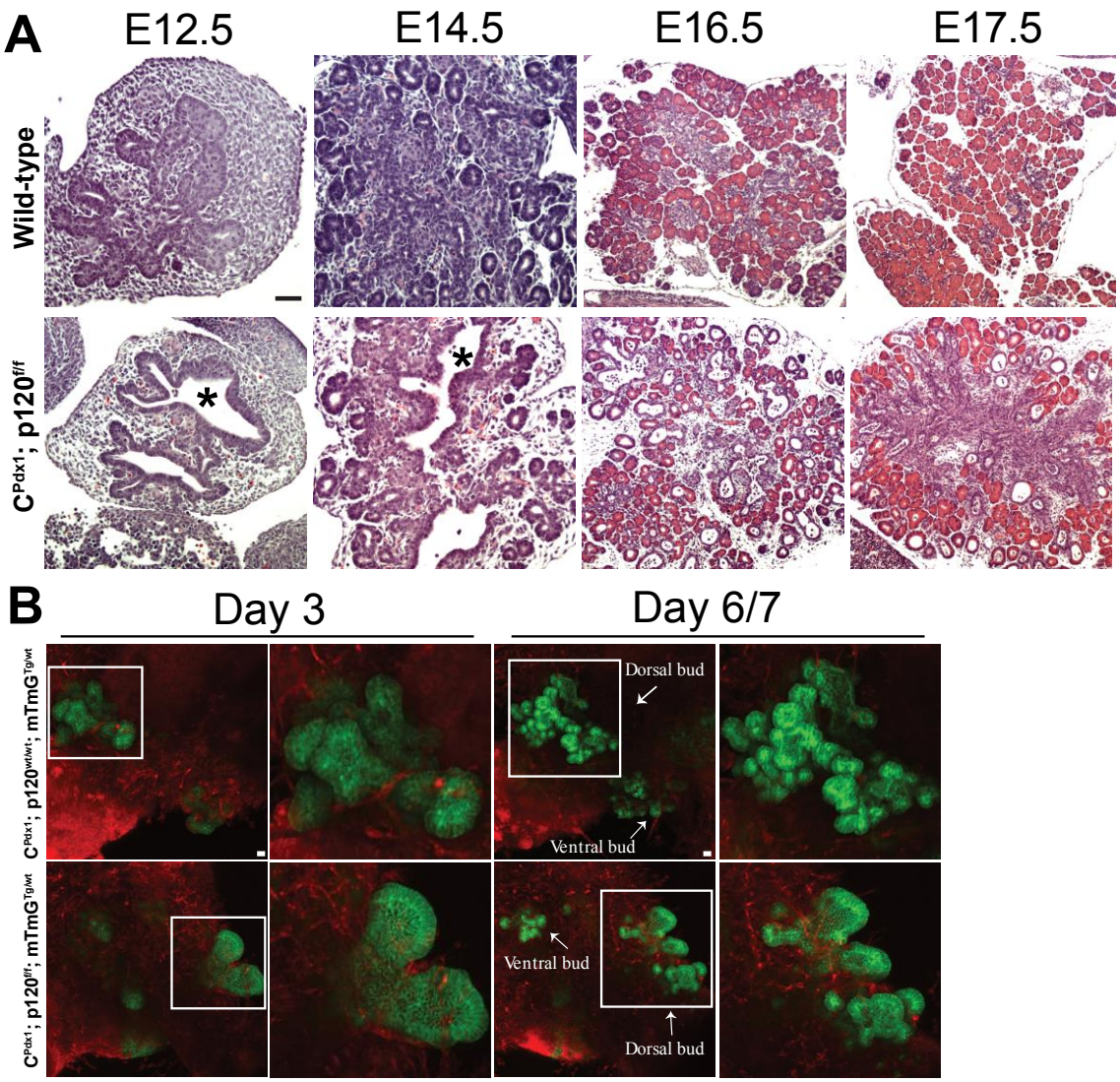

Figure 2.9. Developmental analysis of the p120 catenin loss-of-function phenotype. Hematoxylin and eosin staining at various time points during pancreatic development demonstrated a manifest phenotype as early as E12.5 in $C^{Pdx1}; p120^{f/f}$ animals. (A) p120 catenin loss resulted in expansion of epithelial tubule lumen diameter, decreased ramification of branching tubules, and defects in distribution of all pancreatic cell types during development. Asterisks indicate dilated lumens in $C^{Pdx1}; p120^{f/f}$ pancreases. (B) $C^{Pdx1}; p120^{f/f}; mTmG^{Tg/wt}$ pancreatic bud explants dissected at E11.5 and placed in *in vitro* culture displayed aberrant tubulogenesis, evident as early as Day 3 of culture, suggesting that aberrant tubulogenesis occurred in the absence of associated inflammation. White boxes surround the dorsal pancreatic buds, which are displayed as higher magnification images. White arrows depict the ventral and dorsal pancreatic buds on Day 6/7. Scale bars are 50µm.

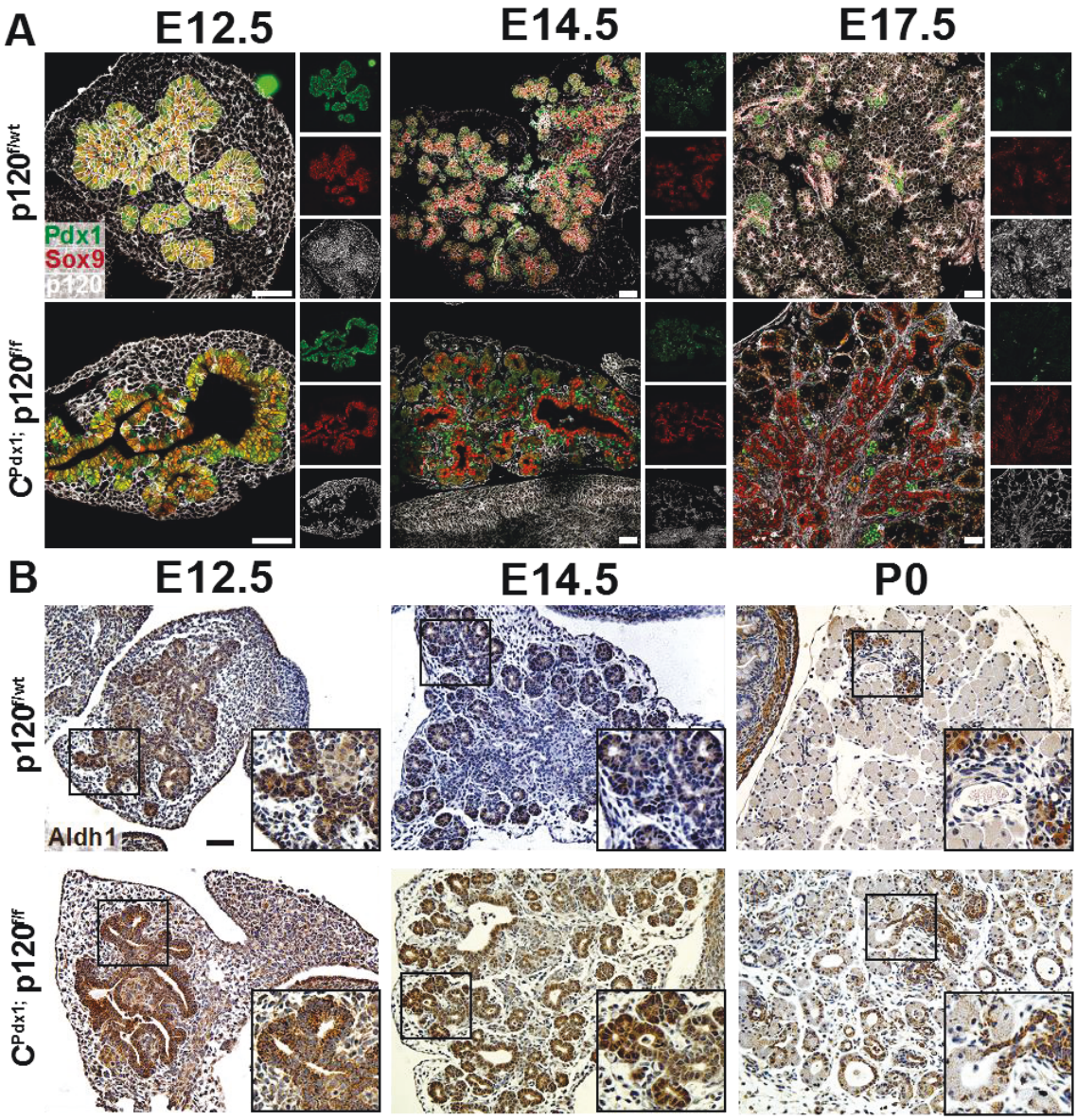

Figure 2.10. Expression of multipotent pancreatic progenitor markers Sox9, Pdx1, and Aldh1. (A) Immunofluorescent images showed expression of early pancreatic progenitor markers Pdx1 and Sox9. The expression pattern of Pdx1 was comparable in both wild-type and homozygous $p120^{f/f}$ pancreases throughout development, while the normal downregulation of Sox9 expression failed to occur in the absence of p120 catenin. (B) Immunohistochemical labeling for Aldh1 demonstrated persistent expression in expanded tubular pancreatic epithelium lacking p120 catenin. Scale bars are 50μm.

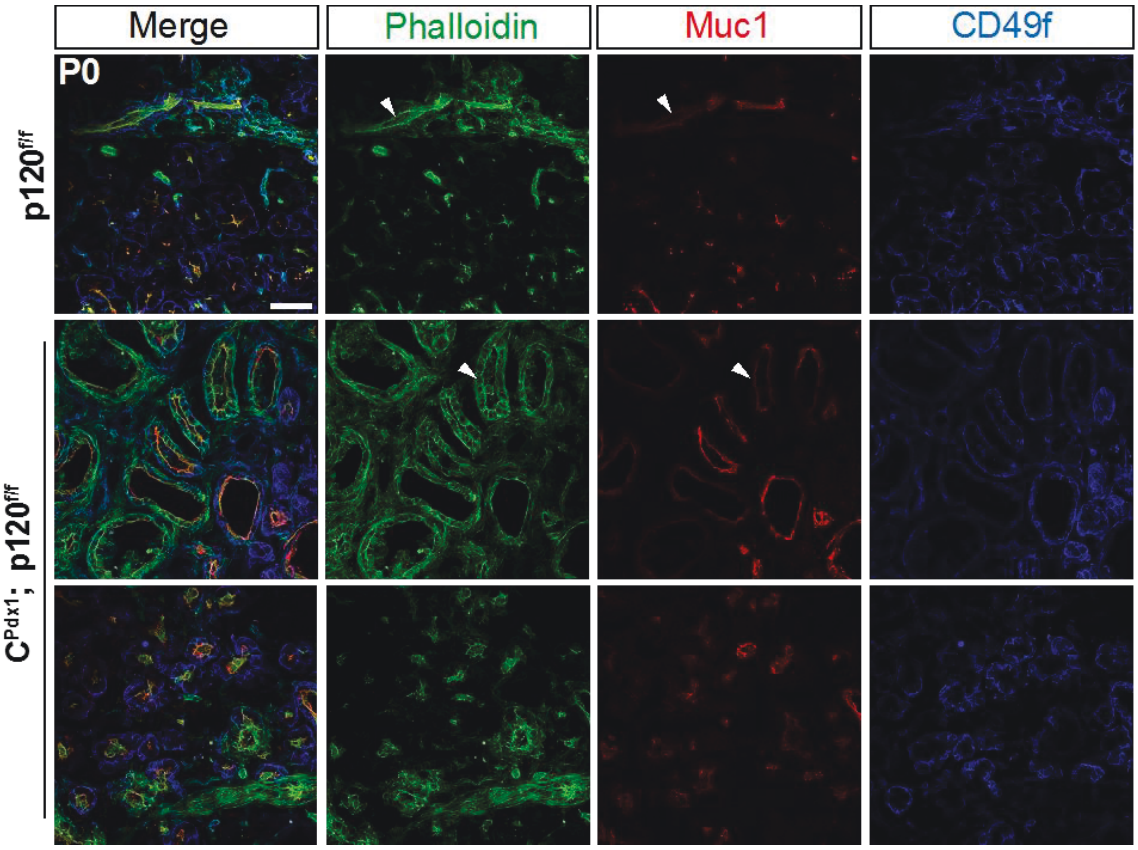

Figure 2.11. Changes in cytoskeletal architecture in pancreatic epithelium lacking p120 catenin. Phalloidin was localized apically in wild-type ducts and apically, laterally, and basally in homozygous *p120$^{f/f}$* ducts, indicating an alteration in cytoskeletal organization. Phalloidin was localized apically in the acini of both homozygous *p120$^{f/f}$* and wild-type control pancreases. Muc1 was localized apically in both wild-type and homozygous *p120$^{f/f}$* pancreatic epithelium. White arrows point to Phalloidin and Muc1 staining in both wild-type and homozygous *p120$^{f/f}$* ducts. The basal marker CD49f has comparable basal localization in wild-type and *C^{Pdx1}; p120$^{f/f}$* pancreatic epithelium. Scale bars are 50µm.

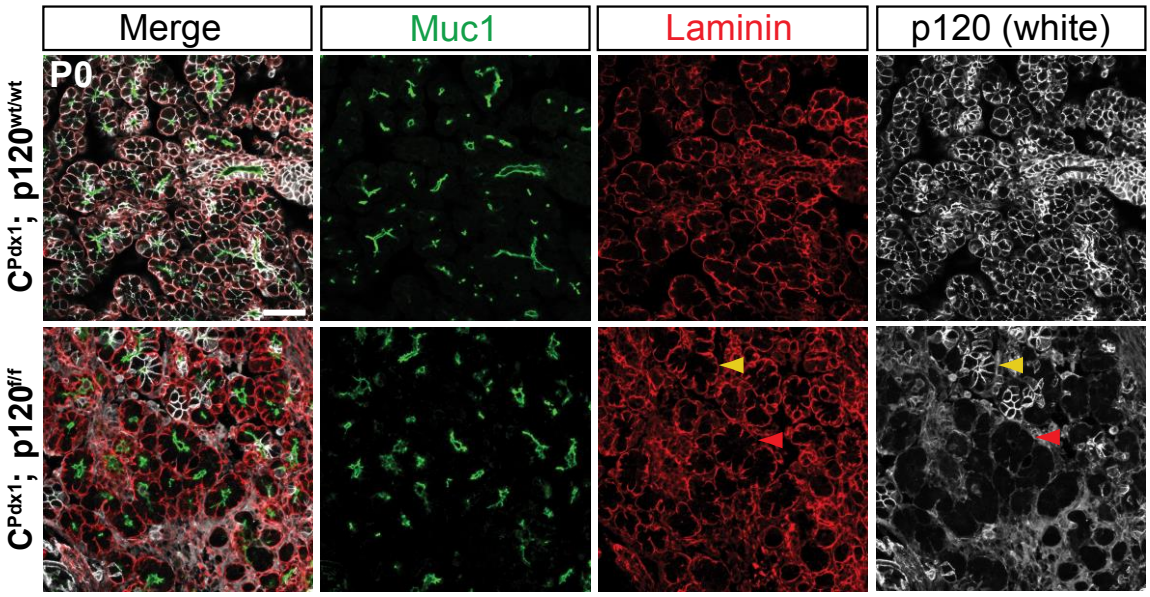

Figure 2.12. Basement membrane Laminin was maintained in *C^{Pdx1};p120$^{f/f}$* pancreatic epithelium. A section with unusually high mosaicism for p120 catenin was intentionally chosen to allow comparison of Muc1 and Laminin staining in p120 catenin-expressing and p120 catenin-deleted tissue in the same section. Yellow arrows point to a p120 catenin-expressing acinus and red arrows show a p120 catenin-deleted acinus. There was no difference in Laminin or Muc1 staining between p120 catenin-expressing and p120 catenin-deleted tissue. Scale bars are 50µm.

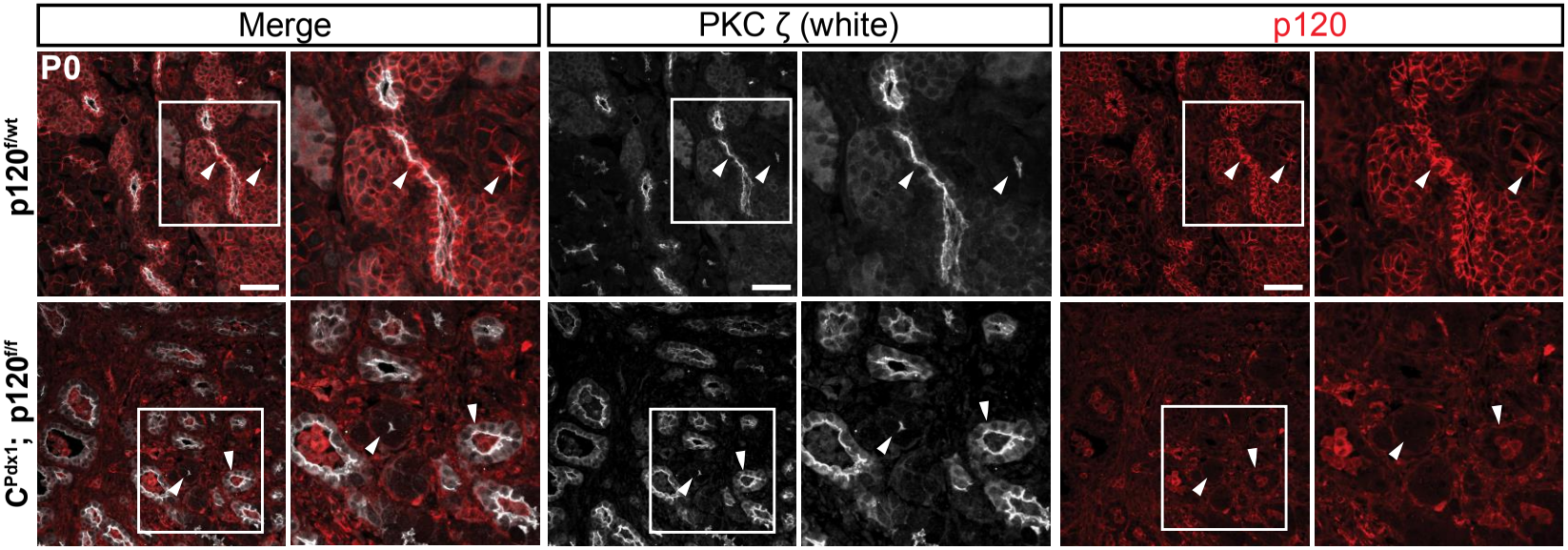

Figure 2.13. C^{Pdx1}; $p120^{f/f}$ pancreatic ductal epithelium displays differential PKCζ localization. In the wild-type panel, white arrows point to apical localization of PKCζ in ductal epithelium (left arrow) and in an acinus (right arrow). In the $C^{Pdx1}; p120^{f/f}$ panel, white arrows point to apical localization of PKCζ in an acinus (left arrow) and both apical and increased cytoplasmic localization of PKCζ in expanded ductal epithelium (right arrow). Higher magnification images are shown for each panel. Scale bars are 50µm.

p120 catenin-null pancreas retains adherens junctions during development but not in adulthood

It was striking to observe that epithelial integrity remained intact during development in $C^{Pdx1}; p120^{f/f}$ pancreata. This was surprising because biochemical studies have demonstrated that loss of p120 catenin results in a failure to retain E-cadherin at epithelial cell membranes (Ireton et al., 2002). This would predict a loss of adherens junction integrity and compromised epithelial cell-cell adhesion. In order to examine adherens junction integrity, we next evaluated localization of members of the adherens junction complex during pancreatic development.

p120 catenin-null pancreatic epithelia display an overall reduction in adherens junction components during development when compared to wild-type controls. However, despite the loss of p120 catenin in pancreatic epithelium, significant amounts of E-cadherin, β-catenin, and α-catenin remained detectable at the membrane throughout embryonic development as assessed by IF (Figure 2.14 A,B and data not shown). The reduction of adherens junction proteins at cell membranes is particularly evident in homozygous $p120^{f/f}$ animals containing mosaic tissue that display both p120 catenin-null and p120 catenin-expressing pancreatic epithelium in the same section (Figure 2.14 A,B).

As an additional means to assess expression of adherens proteins, quantitative RT-PCR was used to compare expression of Cdh1, Ctnnb1, and Ctnna1 between wild-type and $C^{Pdx1}; p120^{f/f}$ pancreata at E14.5 and E17.5. At E14.5, there is no difference in expression of Cdh1 or Ctnnb1, but Ctnna1 expression is significantly increased in homozygous $p120^{f/f}$ pancreata when compared to controls (Figure 2.15A). At E17.5, there is a very significant reduction in expression of Cdh1 and Ctnnb1 in $C^{Pdx1}; p120^{f/f}$

pancreata when compared to wild-type and no difference in expression of Ctnna1 (Figure 2.15B). It is worth noting that although RNA was collected from whole pancreas (epithelium + mesenchyme), we did not observe any difference in the amount of p120 catenin-expressing mesenchyme in homozygous $p120^{f/f}$ animals vs. controls during pancreatic development, which allows us to attribute any differences observed in gene expression directly to pancreatic epithelium. Collectively, these results suggest that despite p120 catenin loss, there remains significant expression of Cdh1, Ctnnb1, and Ctnna1 in pancreatic epithelium during development.

In contrast to the maintenance of organized adherens junctions during development, adult $C^{Pdx1}; p120^{f/f}$ pancreata do not retain E-cadherin and β-catenin at cell membranes in epithelial cells lacking p120 catenin (Figure 2.8C,D). These data suggest that there are compensatory mechanisms present during pancreatic development, but not in adulthood, that permit retention of adherens junctions in the absence of p120 catenin. Nuclear labeling of β-catenin was minimal and comparable in both wild-type and homozygous $p120^{f/f}$ pancreata, as assessed by high resolution confocal microscopy, suggesting that decreased localization of β-catenin to the membrane was not accompanied by an increase in translocalization of β-catenin to the nucleus in homozygous $p120^{f/f}$ pancreata (data not shown).

Because p120 catenin has been shown to play a crucial role in cadherin molecule stability and regulation of cadherin turnover at cell membranes (Davis et al., 2003; Ireton et al., 2002), we next sought to understand the retention of adherens junction members in p120 catenin-null epithelia *in vivo.* We examined the expression of related p120 catenin family members in normal and p120 catenin-deleted pancreas by IF and qPCR. In wild-

type pancreata, ARVCF, δ-catenin, and p0071 were localized uniformly along cell membranes of islet and ductal cells, and at the apical surface of some acini (Figure 2.17A,B,C). δ-catenin was also expressed in some lateral acinar cell membranes in wild-type pancreas (Figure 2.17B). We found that all of the p120 catenin family members examined were present at cell membranes in both p120 catenin-null ducts and acinar cells. Quantitative RT-PCR showed a significant reduction in expression of ARVCF, δ-catenin, and p0071 in homozygous $p120^{f/f}$ pancreata when compared to controls (Figure 2.17D). Retention of E-cadherin, which might be stabilized by substitution of ARVCF, δ-catenin, and p0071 for p120 catenin, likely provides stability for the maintenance of other adherens junctions members and accompanying epithelial integrity during development in $C^{Pdx1}; p120^{f/f}$ animals.

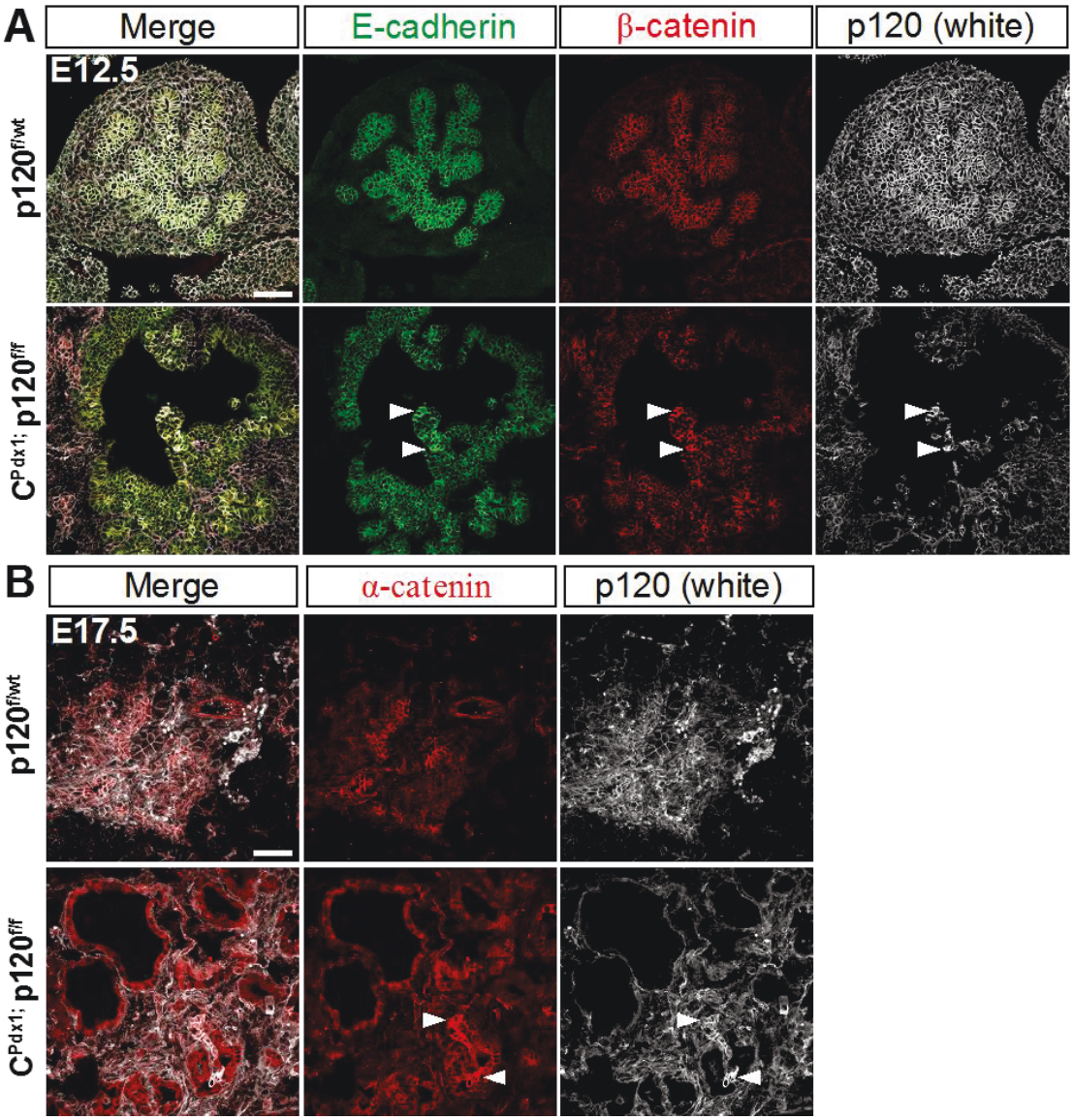

Figure 2.14. Adherens junction components are retained in p120 catenin null epithelia during development. (A-B) Comparison of immunofluorescent staining of p120 catenin, E-cadherin, β-catenin, and α-catenin using embryonic tissues selected for the presence of mosaic p120 catenin-expressing epithelial cells revealed reduced levels but persistent presence of adherens junction components at cell membranes of epithelial cells lacking p120 catenin. White arrows point to a few p120 catenin-expressing epithelial cells in a largely p120 catenin-deleted pancreatic epithelium. Scale bars are 50μm.

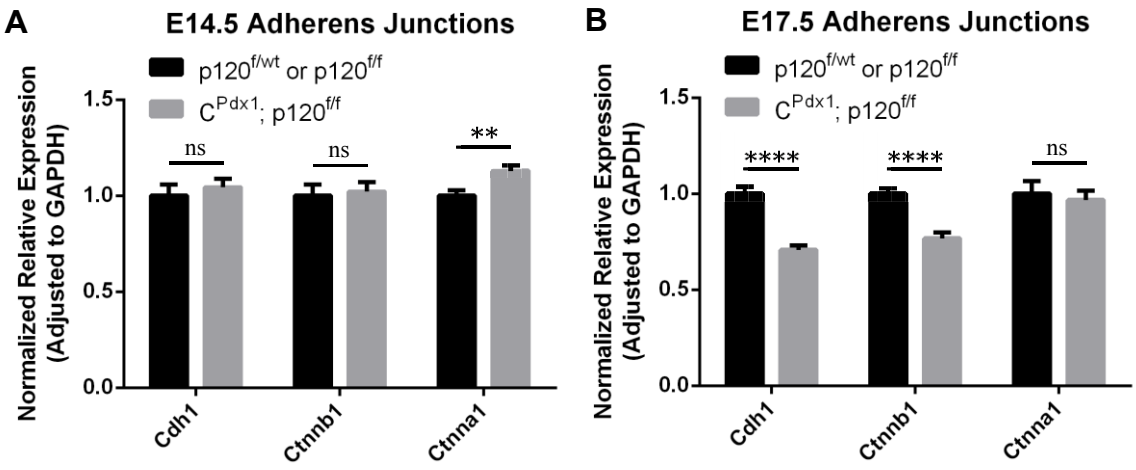

Figure 2.15. Adherens junction components are expressed in *C^{Pdx1};p120$^{f/f}$* epithelia during development. (A-B) Comparison of gene expression of Cdh1, Ctnnb1, and Ctnna1 between wild-type and homozygous *p120$^{f/f}$* pancreata at E14.5 and E17.5 using qPCR. Note that expression of Cdh1 and Ctnnb1 was not significantly different at E14.5 but was reduced at E17.5 in homozygous *p120$^{f/f}$* pancreases when compared to controls. Wild-type control targets were normalized to 1. For all genotypes and all genes at both E14.5 and E17.5 time points, n=7, and reactions were run in quadruplicate. For interpretation of statistical results from Student's t test, ns = not significant and p value > 0.05, * = p value ≤ 0.05, ** = p value ≤ 0.01, *** = p value ≤ 0.001, and **** = p value ≤ 0.0001.

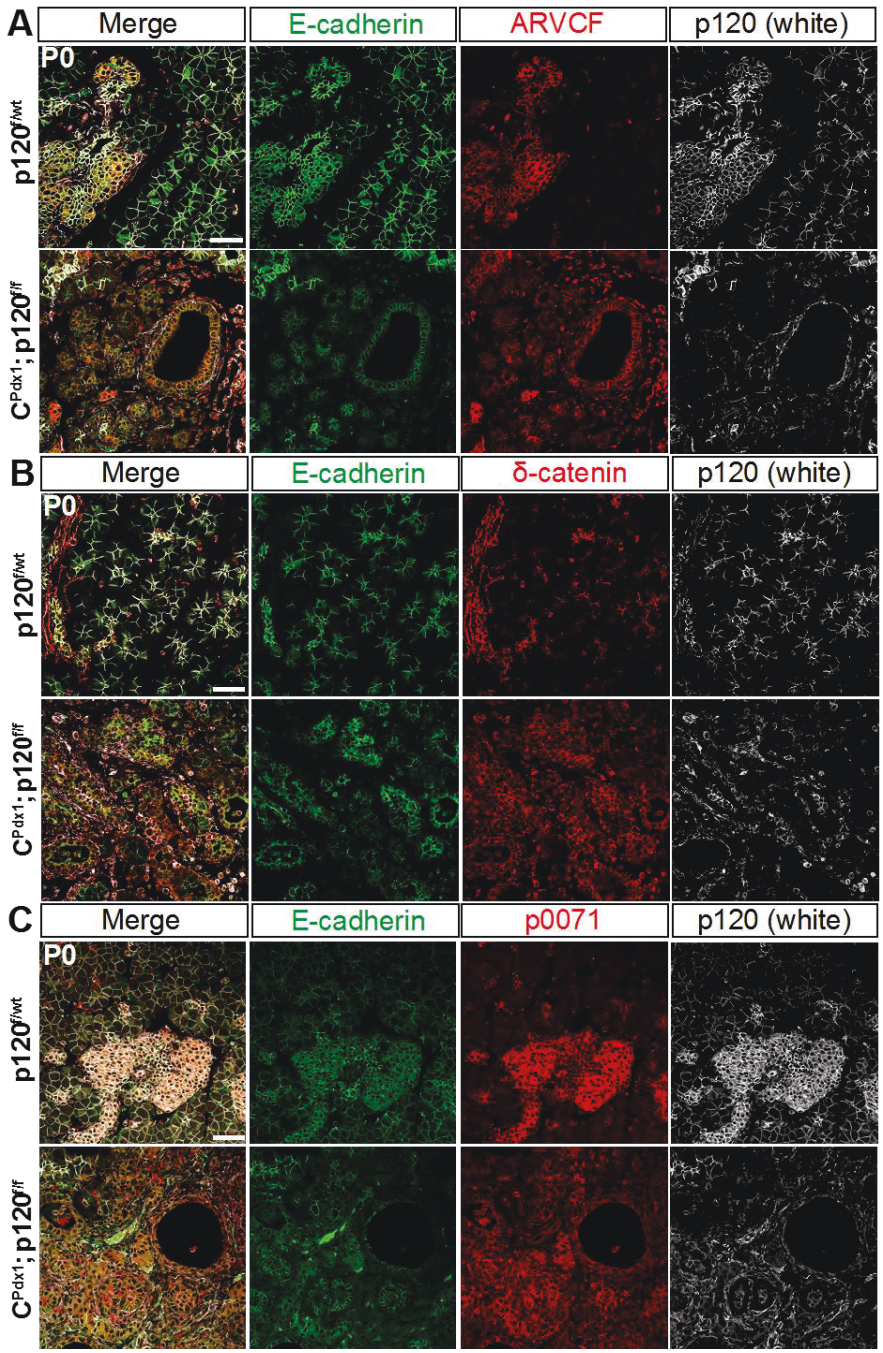

Figure 2.16. p120 catenin family members localize to the membranes of epithelial cells in the absence of p120 catenin. Immunofluorescent images demonstrating expression of p120 catenin family members in wild-type and homozygous *p120$^{f/f}$* pancreas. (A) ARVCF is expressed uniformly in the membranes of duct and islet cells and apically in some acinar cells in wild-type pancreas. It is also localized to the membranes of expanded duct cells and residual acinar cells in homozygous *p120$^{f/f}$* pancreas. (B) δ-catenin is expressed uniformly in the membranes of duct and islet cells and also in the apical and lateral membranes of some acinar cells in wild-type pancreas. In homozygous *p120$^{f/f}$* pancreas, δ-catenin localized to the membranes of expanded ducts. (C) p0071 was expressed uniformly in the membranes of duct and islet cells in wild-type pancreas. Expression of p0071 was also seen uniformly in the membranes of duct cells and remaining acinar cells in homozygous *p120$^{f/f}$* pancreas. Scale bars are 50µm.

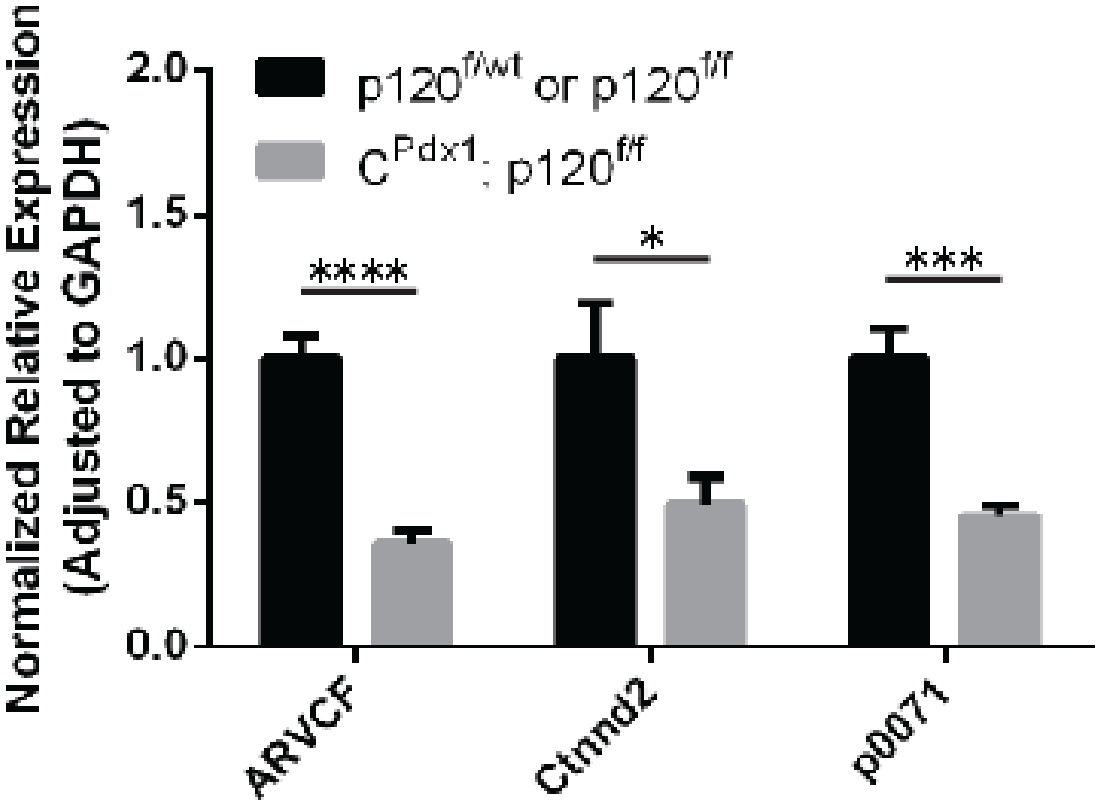

Figure 2.17. p120 catenin family members are expressed in homozygous *p120$^{f/f}$* pancreas. Expression of ARVCF, δ-catenin, and p0071 in *C^{Pdx1}; p120$^{f/f}$* and wild-type pancreases was compared using qPCR. A significant reduction in expression of ARVCF, δ-catenin, and p0071 was observed in homozygous *p120$^{f/f}$* pancreases when compared to controls. Wild-type control targets were normalized to 1. For all genotypes and all genes, n=7, and reactions were run in quadruplicate. For interpretation of statistical results from Student's t test, ns = not significant and p value > 0.05, * = p value ≤ 0.05, ** = p value ≤ 0.01, *** = p value ≤ 0.001, and **** = p value ≤ 0.0001.

DISCUSSION

The effects of conditional deletion of p120 catenin in different organ systems are highly variable, ranging from no observed phenotype (prostate and ureteric bud) to severe developmental defects (salivary gland, mammary gland, kidney, and other organs) and tumor formation (breast, esophagus, and other organs). We have used a mouse model of conditional p120 catenin deletion in epithelial pancreatic lineages to examine the role of p120 catenin during pancreatic development. Our data show that loss of p120 catenin results in overall defects in pancreatic tubulogenesis, branching morphogenesis, and acinar cell differentiation. We also observed differences in the abundance of specific pancreatic cell types and induction of inflammation within the neonatal pancreas. Strikingly, loss of p120 catenin induced changes in the embryonic pancreas that resembled ADM, a condition in the human pancreas that is present in the setting of either pancreatitis or early pancreatic cancer. p120 catenin functions as a regulator of innate anti-inflammatory responses in a growing number of tissues, which now include pancreas (Hu, 2012). Tissue-specific deletion of p120 catenin in both mouse skin and intestine resulted in endogenous recruitment of immune cells and the release of proinflammatory cytokines (Perez-Moreno et al., 2006; Perez-Moreno, Song, Pasolli, Williams, & Fuchs, 2008; Smalley-Freed et al., 2010; Smalley-Freed et al., 2011). In the epidermis, the immune response induced by p120 loss was mediated partly by upregulation of NF-kB signaling (Perez-Moreno et al., 2006). We show that NF-kB is upregulated in the pancreata of $C^{Pdx1}; p120^{f/f}$ animals, and thus might contribute to the recruitment of an inflammatory environment.

Aberrant tubulogenesis has been previously reported in a mouse model of loss of p120 catenin in the renal mesenchyme during glomerulogenesis (Marciano et al., 2011). In homozygous $p120^{f/f}$ animals, we observed aberrant tubulogenesis and an overall decrease in ramification of pancreatic branches at E12.5. The defects in branching morphogenesis may be a secondary effect of the extended epithelial tubule lumen diameter, or p120 catenin loss may have a direct effect on both branching morphogenesis and tubulogenesis. $C^{Pdx1}; p120^{f/f}$ animals also display abnormal localization of all epithelial pancreatic cell types during embryonic development when compared to wild-type controls. Islets fail to migrate throughout the tissue and instead surround a central cluster of $Vimentin^{+}$ cells. Acinar cells are peripherally located while ductal epithelium is mostly centrally located, but can be found throughout the tissue. Homozygous $p120^{f/f}$ pancreata also show a decrease in normal acinar cell differentiation and an expansion of duct-like epithelium during development. A similar result has been previously reported upon deletion of p120 catenin in the mouse salivary gland, which showed blocked acinar cell differentiation and a corresponding increase in ductal epithelium (Davis & Reynolds, 2006). Aberrant actin cytoskeleton organization in expanded ductal epithelium of homozygous $p120^{f/f}$ pancreata was accompanied by increased cytoplasmic PKCζ, suggesting a connection between modulation of the actin cytoskeleton by PKCζ, specifically in the context of p120 catenin loss.

A reduction of adherens junction members at cell membranes upon conditional ablation of p120 catenin has been reported in many developing organ systems (dental enamel, salivary gland, mammary gland, kidney, skin, and vasculature). Despite a reduction, significant retention of adherens junction components are observed in many of

these developing organ systems upon loss of p120 catenin. Collectively, these *in vivo* data differ from biochemical *in vitro* studies which have shown that loss of p120 catenin results in near complete loss of adherens junction components, thereby suggesting that compensatory mechanism(s) for stabilization of cadherin molecules in the absence of p120 catenin exist *in vivo* in some developing organ systems (Davis et al., 2003). For pancreas, retention of adherens junction components in the absence of p120 catenin is observed only during pancreatic development, and not in adults. We show that p120 catenin family members ARVCF, δ-catenin, and p0071 are present at cell membranes even following p120 catenin deletion; since these family members are also capable of stabilizing E-cadherin, they may partially compensate for p120 catenin loss. Relevant to our *in vivo* observations, ARVCF and δ-catenin have previously been shown to rescue E-cadherin stabilization in the absence of p120 catenin *in vitro (Davis et al., 2003)*. However, to our knowledge, no *in vivo* studies to date have shown full functional redundancy for cadherin stabilization by substitution of ARVCF, δ-catenin, or p0071 for p120 catenin in p120 catenin-deleted tissues. Although p120 catenin family members might partially compensate for retention of adherens junctions with loss of p120 catenin, this compensation is not sufficient to guarantee normal pancreas development, and loss of p120 catenin results in a dramatic phenotype. Taken together, our data indicate a crucial role for p120 catenin in pancreatic tubulogenesis, branching morphogenesis, acinar cell differentiation, and regulation of inflammation in both the developing and adult pancreas.

MATERIALS AND METHODS

Mice

All animal studies were approved by the Animal Care and Use Committee at Johns Hopkins University. Mouse strains used in this study were Tg(Pdx1-cre)89.1Dam (MGI ID: 2684317) (G. Gu, Dubauskaite, & Melton, 2002), Ctnnd1^{tm1Abre} (MGI ID: 3617486) (Davis & Reynolds, 2006), and ROSA$^{mT/mG}$ (MGI ID: 3716464) (Muzumdar, Tasic, Miyamichi, Li, & Luo, 2007). The mice were housed under a 14/10 hour light/dark cycle with free access to food and water.

Genotyping

Ctnnd1^{tm1Abre}, Pdx1-cre, and ROSA$^{mT/mG}$ alleles were maintained by breeding heterozygous mice to C57BL/6J mice. Genotyping was accomplished by PCR or Transnetyx. Primers used to genotype for the Ctnnd1^{tm1Abre} allele were p120 FP (5'-TTTTAGAGCCTCCCACATACAAGC-3') and p120 RP (5'-TCAGCACCCACACAAAGGTTG-3') as previously described (Davis & Reynolds, 2006). Primers used to genotype for the Pdx1-cre allele were Pdx1-FP (5'-GAACTGGGGAGGAAAAGGAG-3') and Cre2-RP (5'-GATGAAGCATGTTTAGCTGG-3'). Primers used to genotype for the ROSA$^{mT/mG}$ allele were Rosa26r FP (5'- CTCTGCTGCCTCCTGGCTTCT-3'), Rosa26r RP (5'-CGAGGCGGATCACAAGCAATA-3'), and mTmG RP (5'-TCAATGGGCGGGGGTCGTT-3'). Primers used to determine the sex of neonatal mice were designed to amplify the ZFX and ZFY genes as previously described (Valer Carstea et al., 2007).

Histology/Immunofluorescence

Mouse pancreata were fixed in 10% Neutral Buffered Formalin or 4% Paraformaldehyde at 4°C and embedded in paraffin for sectioning. Five micron sections were prepared for hematoxylin and eosin staining, alcian blue staining, immunofluorescence, and immunohistochemistry. Primary antibodies and other immunofluorescent reagents used in this study are listed in Table 2.1. Secondary antibodies were used at 1:250 and were from Jackson ImmunoResearch.

Embryonic tissues prepared for frozen sections were fixed in 4% Paraformaldehyde at 4°C, subsequently incubated in 30% sucrose at 4°C for cryoprotection, and embedded in OCT (Sakura Finetek 4583 CRYO-OCT Compound). Frozen sections were used for Phalloidin, CD49f, and Muc1 staining in Fig. 5A and α-catenin and p120 catenin staining in Fig. 6B. A citrate-based Antigen Unmasking Solution from Vector Laboratories (H-3300) was used for antigen retrieval for all immunofluorescent staining except in OCT embedded sections. Primary antibody epitopes were retrieved with a heat-mediated microwave antigen retrieval method. All sections were blocked in 10% FBS with 0.2% Triton-X 100 in PBS. Primary antibodies were incubated overnight in blocking buffer at 4°C. Subsequently, secondary antibodies were incubated at RT for 2 hours. Immunofluorescent images were visualized on a Nikon A1 confocal microscope system. Histology of adult $C^{Pdx1}; p120^{f/f}$ males was examined by a pathologist.

Immunohistochemistry

Antigen retrieval for Aldh1 immunohistochemistry was accomplished using Retrievit 6 (BS-1006-00, BioGenex). For Cytokeratin 19 immunolabeling, antigen retrieval was done by digesting sections with 250 μg/mL proteinase K in 2.5 mmol/L $CaCl_2$ and 10mmol/L Tris-HCl (pH 7.5) for 6 minutes at room temperature. For NF-kB immunohistochemistry, antigen retrieval was accomplished using R-Buffer A (62706-10, Electron Microscopy Sciences). Antigen retrieval for CD45 was accomplished using a citrate based antigen retrieval buffer and a heat-mediated microwave method. Immunohistochemistry staining was visualized on an Olympus BX40 light microscope.

Dissection and in vitro culturing of pancreatic bud explants

Pregnant female mice were sacked on E11.5, and embryos were removed. Pancreatic anlagen including surrounding mesenchyme, caudal stomach, and proximal duodenum were dissected from each embryo and cultured as previously described for up to 7 days (Puri & Hebrok, 2007). Day 0 is defined as the day of dissection. For timed breeding, noon of the day when vaginal plugs were first observed was considered 0.5 days post conception (dpc).

RNA isolation, quantitative RT-PCR, and statistical analysis

RNA was isolated from whole mouse pancreas (epithelium + mesenchyme) at various stages of embryonic development and at a postnatal stage using the RNeasy Mini kit (Qiagen). Reverse transcription was accomplished using QuantiTect Reverse Transcription kit (Qiagen). Complementary DNA was amplified using a 7900HT Fast

Real-Time PCR System and TaqMan Gene Expression Assays (Life Technologies). Statistical calculations were performed using GraphPad Prism (GraphPad software) or Microsoft Excel (Microsoft Office 2013), and data are presented as mean ± SEM. Data were compared between groups using an unpaired Student's t test. Significance was accepted at a p value ≤ 0.05.

Table 2.1. Antibodies and other reagents used in pancreatic development study

Antibody	Concentration		Company	Catalogue Number	Reference
	IF	IHC			
α-Amylase	1:500		Sigma-Aldrich	A8273	
α-catenin	1:700		Sigma-Aldrich	C2081	
Aldh1		1:500	Abcam	ab23375	
ARVCF	1:100		Gift from Ilse Hofmann	N/A	(Walter, Schlechter, Hergt, Berger, & Hofmann, 2008)
β-catenin	1:800		BD Transduction Laboratories	610154	
CD45		1:1000	Abcam	ab10558	
CD49f	1:200		BD Biosciences	555734	
Cleaved Caspase-3	1:100		Cell Signaling Technology	9664S	
C-peptide	1:1000		United States Biological	C7905-01R	
Cytokeratin 19		1:100	Developmental Studies Hybridoma Bank	Troma-III	
DBA	1:250		Vector Laboratories	RL-1032	
δ-catenin	1:600		Millipore	07-259	
Ecadherin	1:500		BD Transduction Laboratories	610181	
Ecadherin	1:50		BD Pharmingen	560061	
Glucagon	1:400		Dako	A0565	
Insulin	1:400		Linco	4011-01	
Ki67	1:50		Abcam	ab833	
Laminin	1:50		Abcam	ab11575	
Muc-1 Ab-5	1:200		Calbiochem	HM-1630-P	
NF-kB		1:50	Cell Signaling Technologies	C22B4	
NF-kB		1:50	Abcam	ab28856	
p0071	1:300		Gift from Ilse Hofmann	N/A	(Hofmann, Schlechter, Kuhn, Hergt, & Franke, 2009)
p120 (6H11)	1:1000		Gift from Albert Reynolds	N/A	(Wu, Mariner, Thoreson, & Reynolds, 1998)

p120 F1αSH	1:400		Gift from Albert Reynolds	N/A	(Wu et al., 1998)
Pdx1	1:10000		Gift from Chris Wright	N/A	
Phalloidin	1:100		Invitrogen	A12379	
PKCζ	1:500		Santa Cruz Biotechnology	sc-216	
pp120	1:400		BD Biosciences	610133	
Sox9	1:500		Millipore	AB5535	
Vimentin	1:600		Millipore	AB5733	

CHAPTER 3 – P120 CATENIN SUPPRESSES BASAL EPITHELIAL CELL EXTRUSION IN INVASIVE PANCREATIC NEOPLASIA

INTRODUCTION

Genetic and epigenetic alterations in genes encoding cell adhesion molecules are a hallmark of many epithelial cancers. For pancreas cancer, homophilic cell adhesion has been categorized as one of twelve core signaling pathways (Jones et al., 2008). Homophilic cell adhesion in epithelial cells is mediated partly through cadherins and catenins in adherens junctions. Misexpression of the adherens junction protein p120 catenin has been identified in several types of human carcinomas (van Hengel & van Roy, 2007). Studies have suggested variable roles for p120 catenin in the pathogenesis of epithelial cancers, to include tumor suppression and metastatic progression (Schackmann et al., 2013; Stairs et al., 2011). In human pancreatic cancer, misexpression of p120 catenin in primary tumors is significantly correlated with vascular invasion, metastasis, differentiation, pTNM stage, and poor survival (Fei et al., 2009; Mann et al., 2012; Mayerle et al., 2003). Reduction and cytoplasmic relocalization of p120 catenin has also been reported in 100% of solid pseudopapillary tumors of the pancreas (Chetty, Jain, & Serra, 2008). A study using a forward genetic screen in mice identified *Ctnnd1* as a "candidate cancer gene" in Kras-driven pancreatic neoplasia (Mann et al., 2012). These data suggest that disruption of *CTNND1* in pancreatic tumors has biological relevance to disease, yet, the mechanisms by which p120 catenin contributes to the development and progression of pancreatic cancer are not understood.

Increased occurrence of metastasis with altered p120 catenin expression suggests that p120 catenin may play a role in metastatic progression of pancreatic cancer. A

mechanism recently hypothesized to initiate metastasis by mediating invasion is basal epithelial cell extrusion (Y. Gu et al., 2015; G. M. Slattum & Rosenblatt, 2014). Epithelial tissues maintain homeostatic cell numbers by extruding cells through a highly conserved mechanism involving production and secretion of the signaling lipid sphingosine 1-phosphate (S1P). Extracellular S1P binds to S1P receptor 2 (S1pr2) on neighboring cells, which induces contraction of an actomyosin band that extrudes the cell out of the epithelium while preserving barrier function (Andrade & Rosenblatt, 2011; Eisenhoffer et al., 2012; Eisenhoffer & Rosenblatt, 2013; Y. Gu, Forostyan, Sabbadini, & Rosenblatt, 2011; Y. Gu & Rosenblatt, 2012; Rosenblatt, Raff, & Cramer, 2001; G. Slattum, McGee, & Rosenblatt, 2009). Mutations in the tumor suppressor *APC* and oncogenic Kras have been shown to shift the predominant direction of epithelial cell extrusion from apical to basal, where extruded cells invade the underlying epithelium and survive (Marshall, Lloyd, Delalande, Nathke, & Rosenblatt, 2011; G. Slattum, Gu, Sabbadini, & Rosenblatt, 2014). The results presented in this study show that p120 catenin restrains epithelial cell extrusion in the earliest stages of pancreatic neoplastic invasion, via a S1P/S1pr2-dependent mechanism.

RESULTS

p120 catenin is misexpressed in human premalignant and malignant pancreatic lesions

Expression and localization of p120 catenin, a critical cytoskeletal regulator and component of adherens junctions, were rated in human normal pancreas, chronic pancreatitis, acinar to ductal metaplasia (ADM), pancreatic intraepithelial neoplasia (PanIN) 1-3, and primary tumors and metastases. Examples of scores in each category are presented in Figure 3.1. p120 catenin is expressed in all normal pancreatic cell types (Figure 3.2A,B). Localization of p120 catenin was scored as predominantly membranous in 100% of normal pancreatic cell types (n=22-33 patients for each pancreatic cell type) (Figure 3.3D). Punctate cytoplasmic and nuclear staining were also observed in normal pancreatic cells (Figure 3.2A,B). p120 catenin expression was rated as high in >91% chronic pancreatitis and ADM (n=25/26 chronic pancreatitis, n=23/25 ADM) (Figure 3.2C,I). Expression levels of p120 catenin were significantly decreased in primary tumors when compared to PanIN lesions (n=31 PanIN and n=16 primary tumors, P=0.0005) (Figures 3.2D-F, 3.3A,C). Moreover, p120 catenin expression was significantly lower in metastases when compared to primary tumors (n=16 primary tumors and n=16 metastasis) (P=0.0361) (Figure 3.3A-C). There was also a significant difference in predominant p120 catenin subcellular localization when comparing PanIN1-2 and PanIN3 (n=26 PanIN1-2 and n=5 PanIN3, P=0.0082) (Figure 3.3D). Together, these findings demonstrate that altered p120 catenin expression and localization is a distinguishing hallmark of human pancreatic cancer progression.

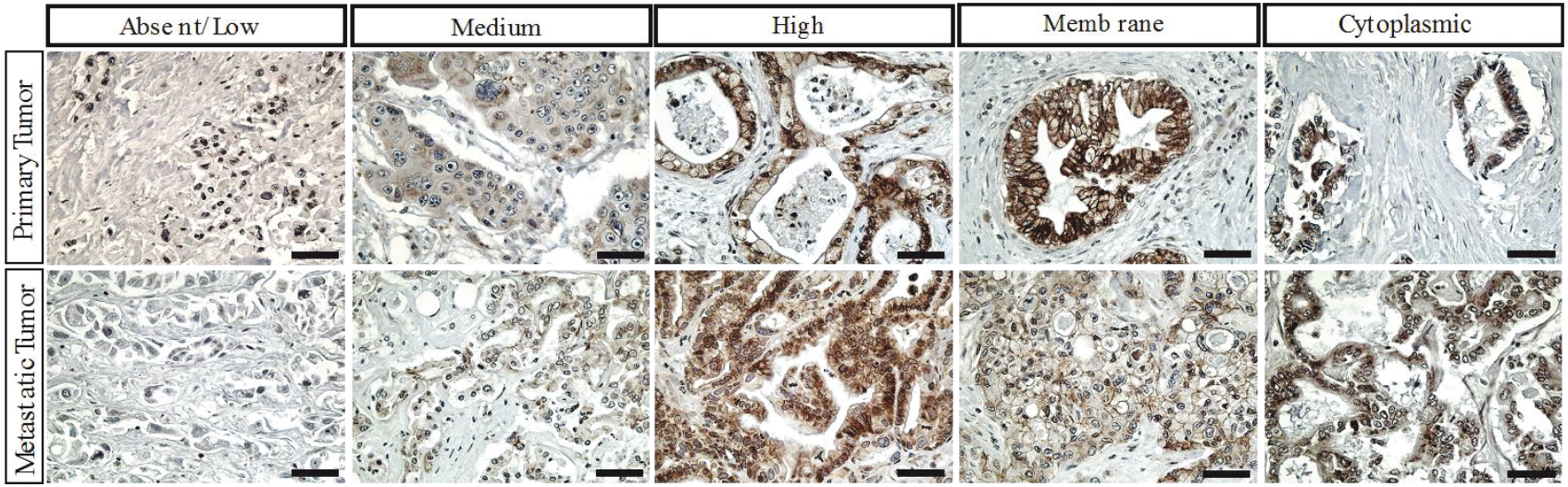

Figure 3.1. Representative images used for scoring p120 catenin expression in pancreatic tissues. For p120 catenin expression and subcellular localization scoring, IHC depicts examples of absent/low, medium, and high p120 catenin expression in primary and metastatic pancreatic tumors. IHC also shows examples of predominant membranous and cytoplasmic p120 catenin subcellular localization. Scale bars are 50μm.

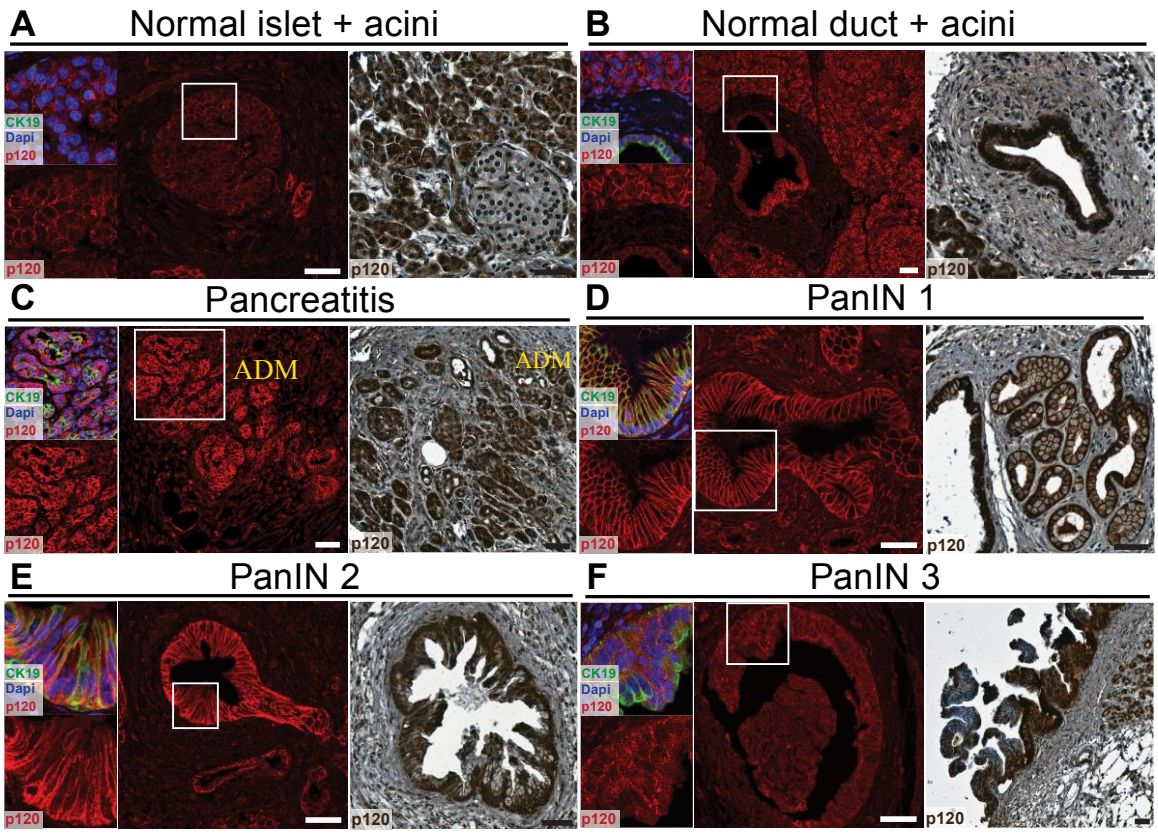

Figure 3.2. Expression of p120 catenin in human pancreas. A,B) Immunohistochemistry (IHC) shows high p120 catenin expression in normal acinar and duct cells and medium p120 catenin expression in normal islets. Immunofluorescence (IF) labeling showing p120 catenin overlaid with CK19 and Dapi allowed visualization of punctate cytoplasmic and nuclear p120 catenin in normal pancreatic cells. C-E) Pancreatitis, ADM, and PanIN1-2 lesions depicted show predominant membranous and high p120 catenin expression. F) PanIN3 depicted shows predominant cytoplasmic and high p120 catenin expression. Scale bars are 50µm.

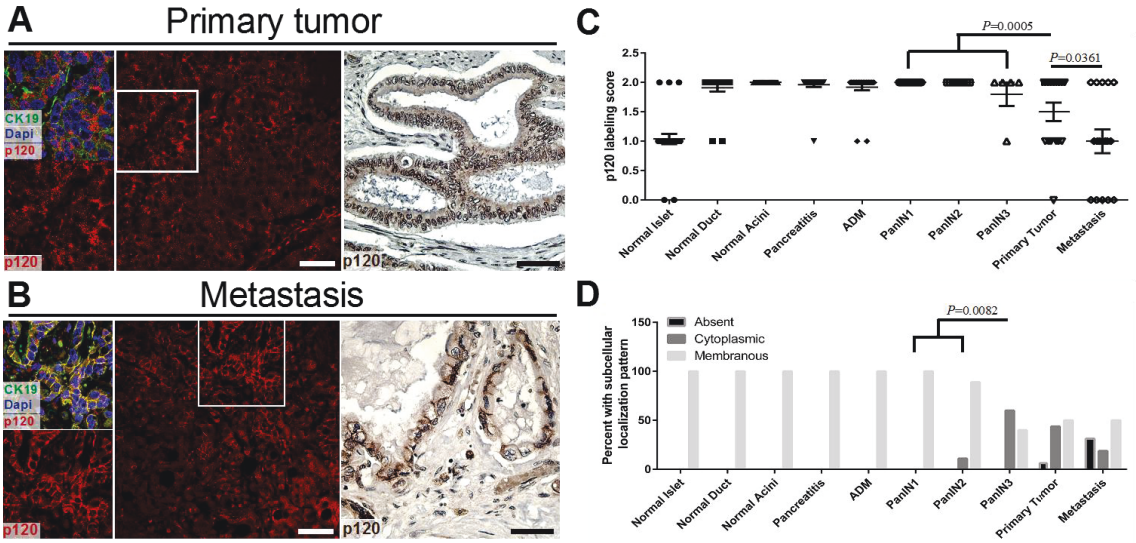

Figure 3.3. Loss of p120 catenin significantly correlates with metastatic pancreatic cancer progression in humans. A) Images of primary pancreatic tumors depicted show predominant cytoplasmic and both absent/low (right) and high (left) p120 catenin labeling. B) The IF metastasis image shows heterogeneous p120 catenin expression with regions containing membranous, cytoplasmic, and absent p120 catenin, and the IHC image shows high, membranous p120 catenin expression. C) Numerical scoring of p120 catenin expression revealed a significant decrease in primary tumors when compared to PanIN1-3 and metastasis when compared to primary tumors. D) Analysis of predominant p120 catenin subcellular localization revealed a significant difference when comparing PanIN1-2 and PanIN3. The P value was calculated using Fisher's exact test. Scale bars are 50μm.

Pancreatic loss of p120 catenin in the context of oncogenic Kras results in accelerated PanIN progression

Previously, using $C^{Pdx1}; p120^{f/f}$ mice, we reported that p120 catenin is required for proper tubulogenesis and cell-type specification during pancreas development (Hendley et al., 2015). To determine the function of p120 catenin in adult mouse pancreas in the absence of a confounding developmental phenotype, we crossed transgenic mice harboring floxed alleles of p120 catenin *(p120^{f})* (Davis & Reynolds, 2006) with $Mist1^{CreER/+}$ *(*C^{iMist1}*)* mice (Habbe et al., 2008). $p120^{f/f}$, $C^{iMist1}; p120^{wt/wt}$, $C^{iMist1}; p120^{f/wt}$, and $C^{iMist1}; p120^{f/f}$ mice displayed normal pancreatic histology 2-4 months post tamoxifen injection (Figure 3.4A-C). Similar to what we previously reported for $C^{Pdx1}; p120^{f/f}$ mice 10 months of age (Hendley et al., 2015), a subset of $C^{iMist1}; p120^{f/f}$ mice 12 months post tamoxifen injection exhibited pancreatitis and ADM (Figure 3.4D).

Since we observed mislocalized p120 catenin in human PanIN, before the onset of pancreatic cancer, we next sought to determine if p120 catenin plays a functional role in PanIN formation and progression. To this end, we crossed $C^{iMist1}; p120^{f/f}$ mice with *lox-stop-lox-*$Kras^{G12D}$ (K) mice (Hingorani et al., 2003), which resulted in simultaneous ablation of p120 catenin and activation of oncogenic $Kras^{G12D}$ in adult pancreatic acinar cells upon tamoxifen administration (Figure 3.5A). The KC^{iMist1} mouse model displays the full spectrum of murine preinvasive ADM and PanIN1-3 lesions in a manner that faithfully recapitulates human preinvasive pancreatic lesions (Habbe et al., 2008). We performed survival analysis on cohorts of $KC^{iMist1}; p120^{wt/wt}$, $KC^{iMist1}; p120^{f/wt}$, and $KC^{iMist1}; p120^{f/f}$ mice, which showed significant differences in overall survival (Figure 3.5B). $KC^{iMist1}; p120^{f/f}$ mice exhibited cachexia, which is also frequently observed in

human pancreatic cancer patients (Figure 3.5C,D). The gross appearance of *KC^{iMist1}; p120$^{f/f}$* pancreata was strikingly abnormal and enlarged when compared to *KC^{iMist1}; p120$^{wt/wt}$* pancreata (Figure 3.5E-G).

Histologically, *KC^{iMist1}; p120$^{wt/wt}$*, *KC^{iMist1}; p120$^{f/wt}$*, and *KC^{iMist1}; p120$^{f/f}$* pancreata are comparable at 1 week post tamoxifen injection (Figure 3.6A,A'). At 2 weeks post tamoxifen injection, *KC^{iMist1}; p120$^{f/f}$* pancreata show significantly accelerated ADM formation and increased recruitment of a fibroinflammatory infiltrate when compared to *KC^{iMist1}; p120$^{wt/wt}$* and *KC^{iMist1}; p120$^{f/wt}$* pancreata (Figure 3.6B,B'). One month post tamoxifen injection, *KC^{iMist1}; p120$^{f/f}$* pancreata display significantly increased fibrostroma (92.89% ± 0.66% pancreatic area, n=6 mice) when compared to *KC^{iMist1}; p120$^{f/wt}$* pancreata (0.14% ± 0.03% pancreatic area, n=4 mice) (P=<0.0001) and *KC^{iMist1}; p120$^{wt/wt}$* pancreata (0.31% ± 0.21% pancreatic area, n=6 mice) (P=<0.0001). Significant increases in low and high grade PanIN formation were observed in *KC^{iMist1}; p120$^{f/f}$* pancreata when compared to *KC^{iMist1}; p120$^{f/wt}$* and *KC^{iMist1}; p120$^{wt/wt}$* pancreata (Figure 3.7A,A',B,B').

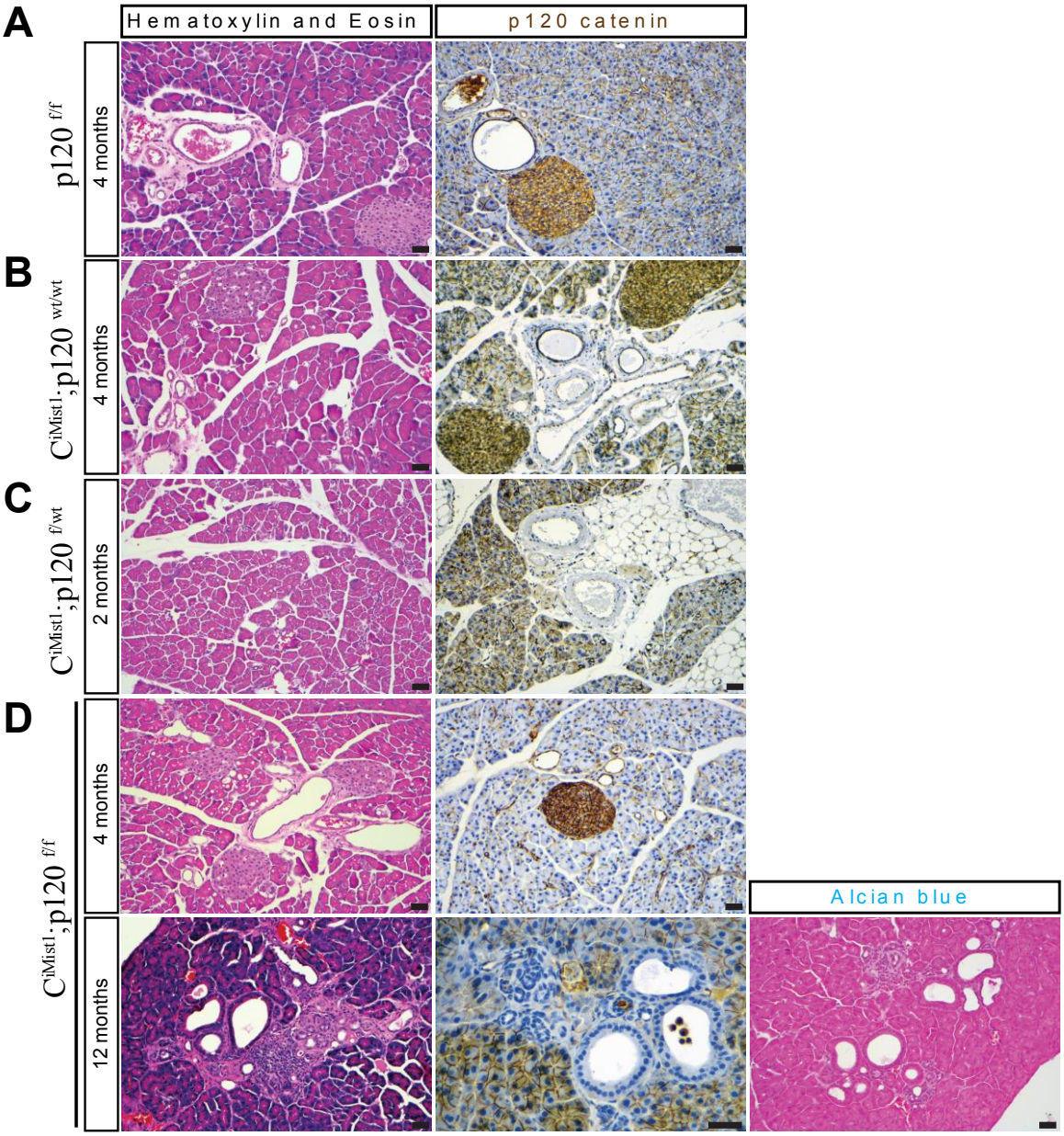

Figure 3.4. *C^{iMist1}; p120$^{f/f}$* pancreata show ADM and pancreatitis. A-D) *p120$^{f/f}$*, *C^{iMist1}; p120$^{wt/wt}$*, and *C^{iMist1}; p120$^{f/wt}$* pancreata displayed normal histology, and IHC showed p120 catenin expression at 2-4 months of age. E) *C^{iMist1}; p120$^{f/f}$* pancreata 4 months of age showed normal histology and lack p120 catenin expression in acinar cells. *C^{iMist1}; p120$^{f/f}$* pancreata 12 months of age developed ADM and pancreatitis (n=1/3 mice). Note that the 12 month image contains unusually high mosaic p120 catenin expression in acinar cells, but ADM lack p120 catenin expression, suggesting that these lesions may be forming in a cell autonomous manner. We stained for Alcian blue to suggest the presence of murine PanIN, and did not observe blue staining in *C^{iMist1}; p120$^{f/f}$* pancreata. Scale bars are 50μm.

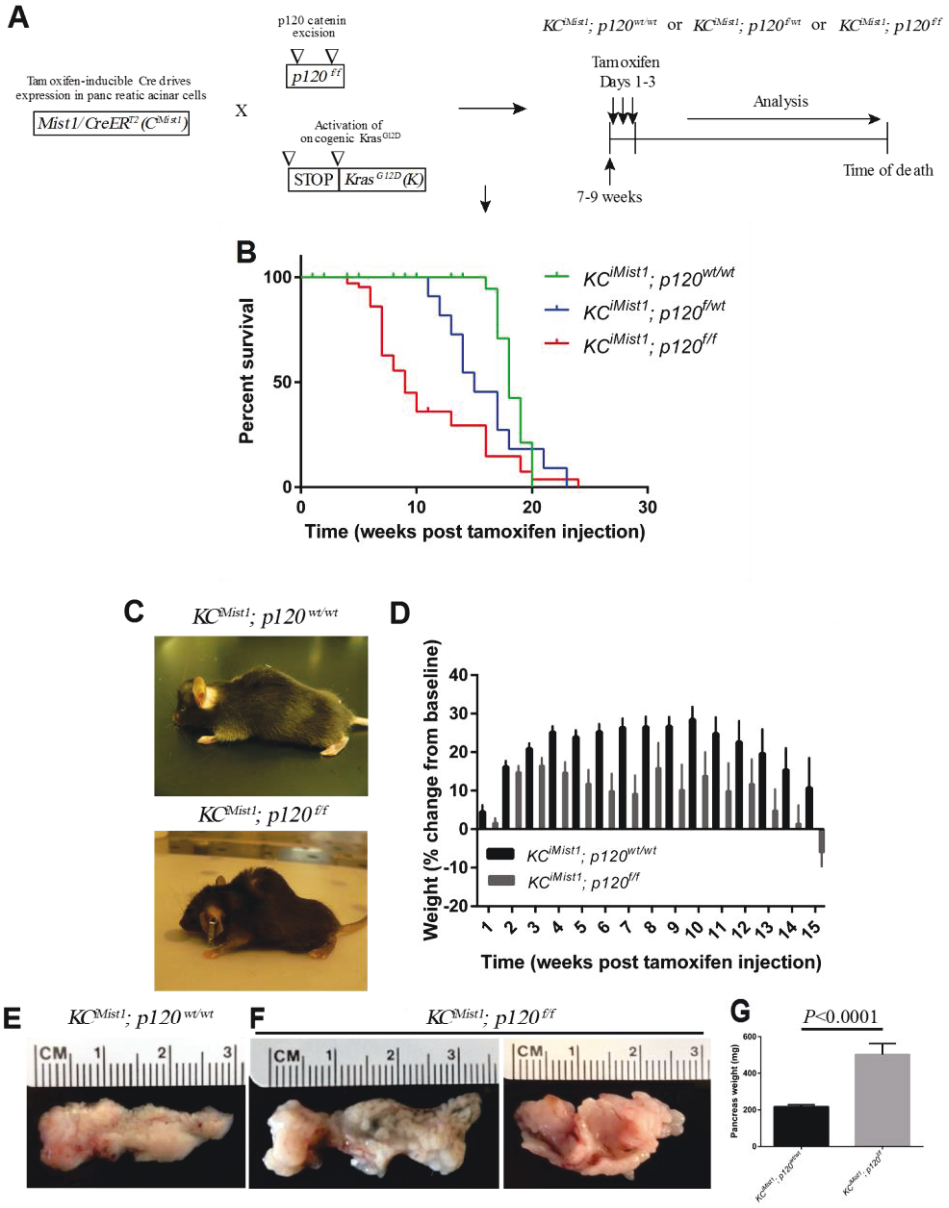

Figure 3.5. Oncogenic Kras and loss of p120 catenin lead to decreased overall survival, cachexia, and increased pancreas size. A) Breeding scheme for generation of *KC^{iMist1}; p120$^{wt/wt}$*, *KC^{iMist1}; p120$^{f/wt}$*, and *KC^{iMist1}; p120$^{f/f}$* mice and tamoxifen administration scheme. B) Kaplan-Meyer overall survival analysis of the indicated mouse genotypes: *KC^{iMist1}; p120$^{wt/wt}$* (green, n=9), *KC^{iMist1}; p120$^{f/wt}$* (blue, n=10), and *KC^{iMist1}; p120$^{f/f}$* (red, n=39). Overall median survival was 18 weeks for *KC^{iMist1}; p120$^{wt/wt}$* mice, 14.5 weeks for *KC^{iMist1}; p120$^{f/wt}$* mice, and 9 weeks for *KC^{iMist1}; p120$^{f/f}$* mice. Log-rank test revealed significantly longer survival of *KC^{iMist1}; p120$^{wt/wt}$* mice when compared to *KC^{iMist1}; p120$^{f/wt}$* (P=0.0118) and *KC^{iMist1}; p120$^{f/f}$* (P<0.0001) mice. C) A *KC^{iMist1}; p120$^{f/f}$* mouse sacrificed near death at 9 weeks post tamoxifen injection is shown along with a *KC^{iMist1}; p120$^{wt/wt}$* mouse sacrificed 9 weeks post tamoxifen injection. D) *KC^{iMist1}; p120$^{f/f}$* (n=70 total) mice exhibit cachexia when compared to *KC^{iMist1}; p120$^{wt/wt}$* (n=62 total) mice. E,F) 1 month post tamoxifen injection, *KC^{iMist1}; p120$^{f/f}$* (n=13) pancreata have a rigid appearance and are significantly larger than *KC^{iMist1}; p120$^{wt/wt}$* (n=13) pancreata.

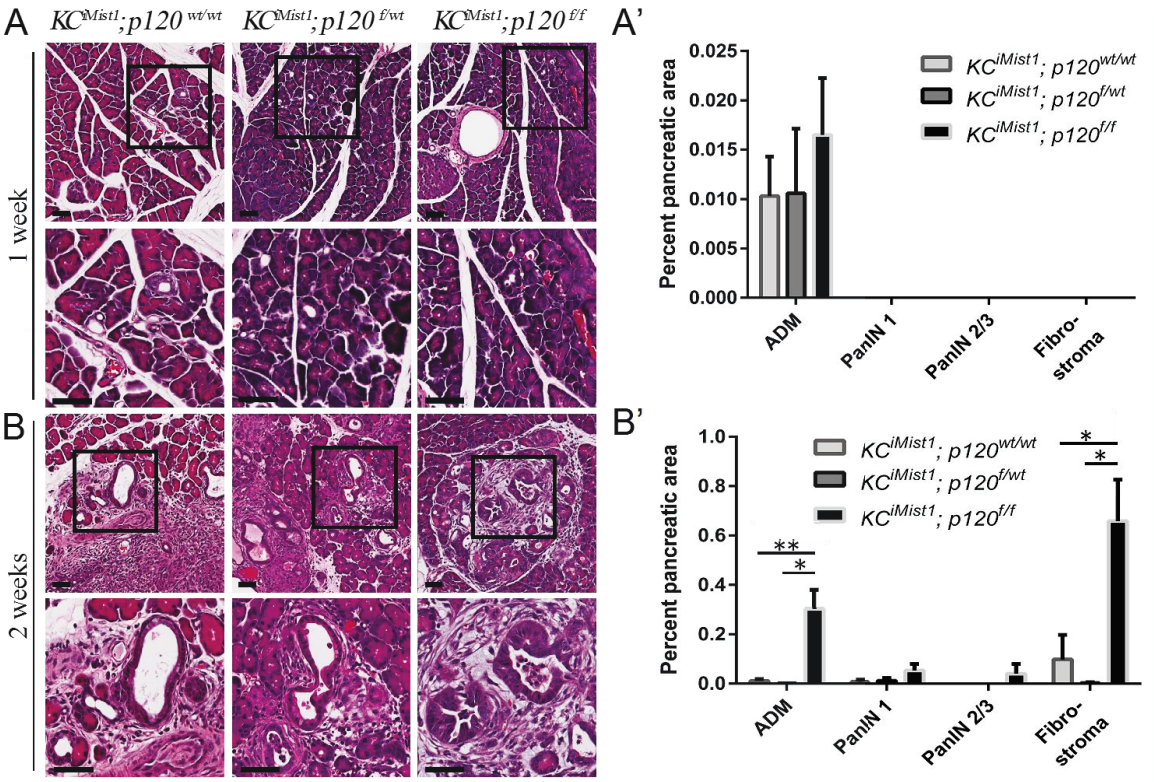

Figure 3.6. p120 catenin restrains formation of Kras-induced preinvasive pancreatic cancer. A,A') 1 week post tamoxifen injection, there is no significant difference in ADM formation when comparing *KC^{iMist1}; p120$^{wt/wt}$* (n=3), *KC^{iMist1}; p120$^{f/wt}$* (n=2), and *KC^{iMist1}; p120$^{f/f}$* (n=3) pancreata. B,B') 2 weeks post tamoxifen injection, *KC^{iMist1}; p120$^{f/f}$* (n=5) pancreata display significantly accelerated ADM formation when compared to *KC^{iMist1}; p120$^{f/wt}$* (n=2) (*P*=0.0479) and *KC^{iMist1}; p120$^{wt/wt}$* (n=5) (*P*=0.0053) pancreata. In addition, *KC^{iMist1}; p120$^{f/f}$* pancreata display significantly increased fibrostroma when compared to *KC^{iMist1}; p120$^{f/wt}$* (*P*=0.0392) and *KC^{iMist1}; p120$^{wt/wt}$* (*P*=0.0207) pancreata. Scale bars are 50μm.

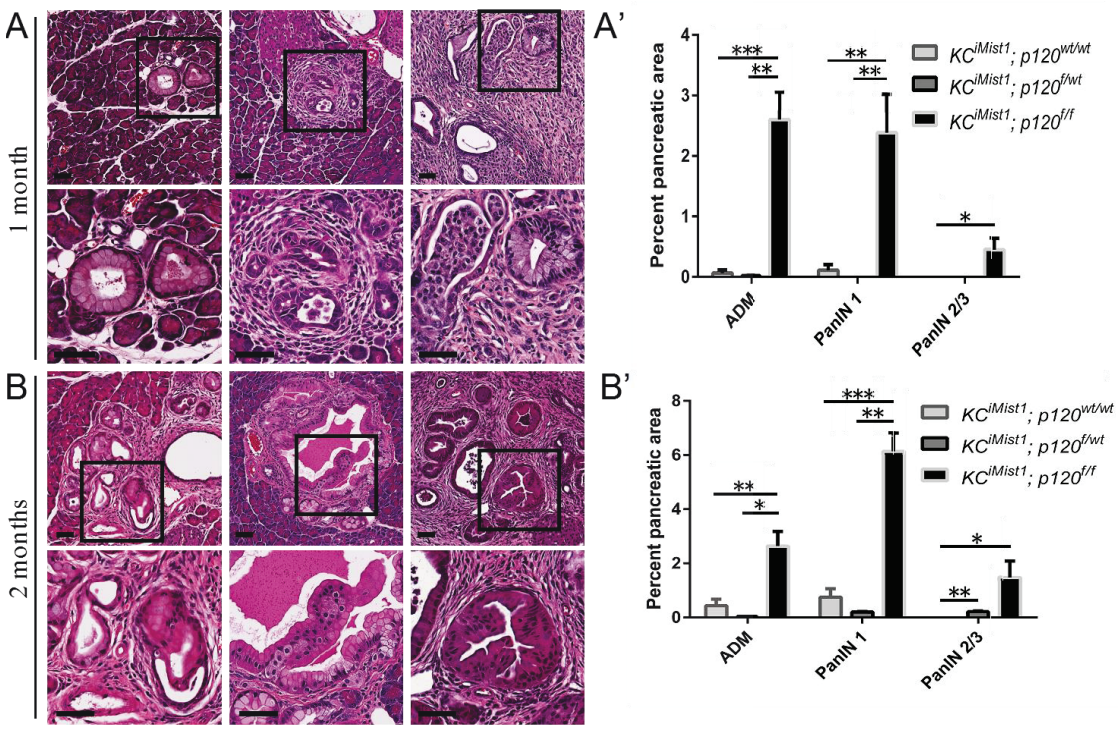

Figure 3.7. p120 catenin loss accelerates formation of premalignant pancreatic cancer. A,A') 1 month post tamoxifen injection, *KC^{iMist1}; p120$^{f/f}$* (n=6) pancreata show significantly increased ADM (*P*=0.0002), PanIN1 (*P*=0.0053), and PanIN2/3 (*P*=0.0415) formation when compared to *KC^{iMist1}; p120$^{wt/wt}$* (n=6) pancreata. *KC^{iMist1}; p120$^{f/f}$* pancreata also display significantly increased ADM and PanIN formation when compared to *KC^{iMist1}; p120$^{f/wt}$* (n=4) pancreata (*P*=0.0017 and *P*=0.017, respectively). B,B') The only significant difference between *KC^{iMist1}; p120$^{wt/wt}$* (n=4) and *KC^{iMist1}; p120$^{f/wt}$* (n=2) pancreata is observed 2 months post tamoxifen injection as increased high grade PanIN formation in *KC^{iMist1}; p120$^{f/wt}$* pancreata (*P*=0.0003). *KC^{iMist1}; p120$^{f/f}$* (n=4) pancreata show significantly increased ADM (*P*=0.0092), PanIN1 (*P*=0.0004), and PanIN2/3 (*P*=0.0498) formation when compared to *KC^{iMist1}; p120$^{wt/wt}$* (n=4) pancreata. *KC^{iMist1}; p120$^{f/f}$* pancreata also display significantly increased ADM (*P*=0.0312) and PanIN1 (*P*=0.0043) formation when compared to *KC^{iMist1}; p120$^{f/wt}$* pancreata. Scale bars are 50μm.

KC^{iMist1}; $p120^{f/f}$ pancreata show a prominent acute and chronic inflammatory response

As expected, p120 catenin was ubiquitously expressed in *KC^{iMist1}; $p120^{wt/wt}$* and *KC^{iMist1}; $p120^{f/wt}$* pancreata (Figure 3.8A,B). Minimal mosaic expression of p120 catenin was observed in *KC^{iMist1}; $p120^{f/f}$* pancreatic acini (Figure 3.8C). *KC^{iMist1}; $p120^{f/f}$* pancreata displayed marked acinar cell atrophy, pronounced inflammation, and contained stroma characterized by a unique cellular constitution that differs from the stroma in *KC^{iMist1}; $p120^{wt/wt}$* pancreata (Figure 3.8D-L). *KC^{iMist1}; $p120^{f/f}$* pancreata displayed less mucinous lesions than *KC^{iMist1}; $p120^{wt/wt}$* and *KC^{iMist1}; $p120^{f/wt}$* pancreata, as manifested by Alcian blue staining (Figure 3.9A-C). *KC^{iMist1}; $p120^{f/f}$* pancreata also showed areas of ductal dilation (Figure 3.10A-C). Disruption of contiguous basement membrane Laminin expression, a characteristic of human pancreatic cancer (Pan et al., 2009), was also seen in *KC^{iMist1}; $p120^{f/f}$* pancreatic lesions. This was accompanied by cells that escaped intact PanIN epithelium and invaded into the underlying tissue (Figure 3.10D-F).

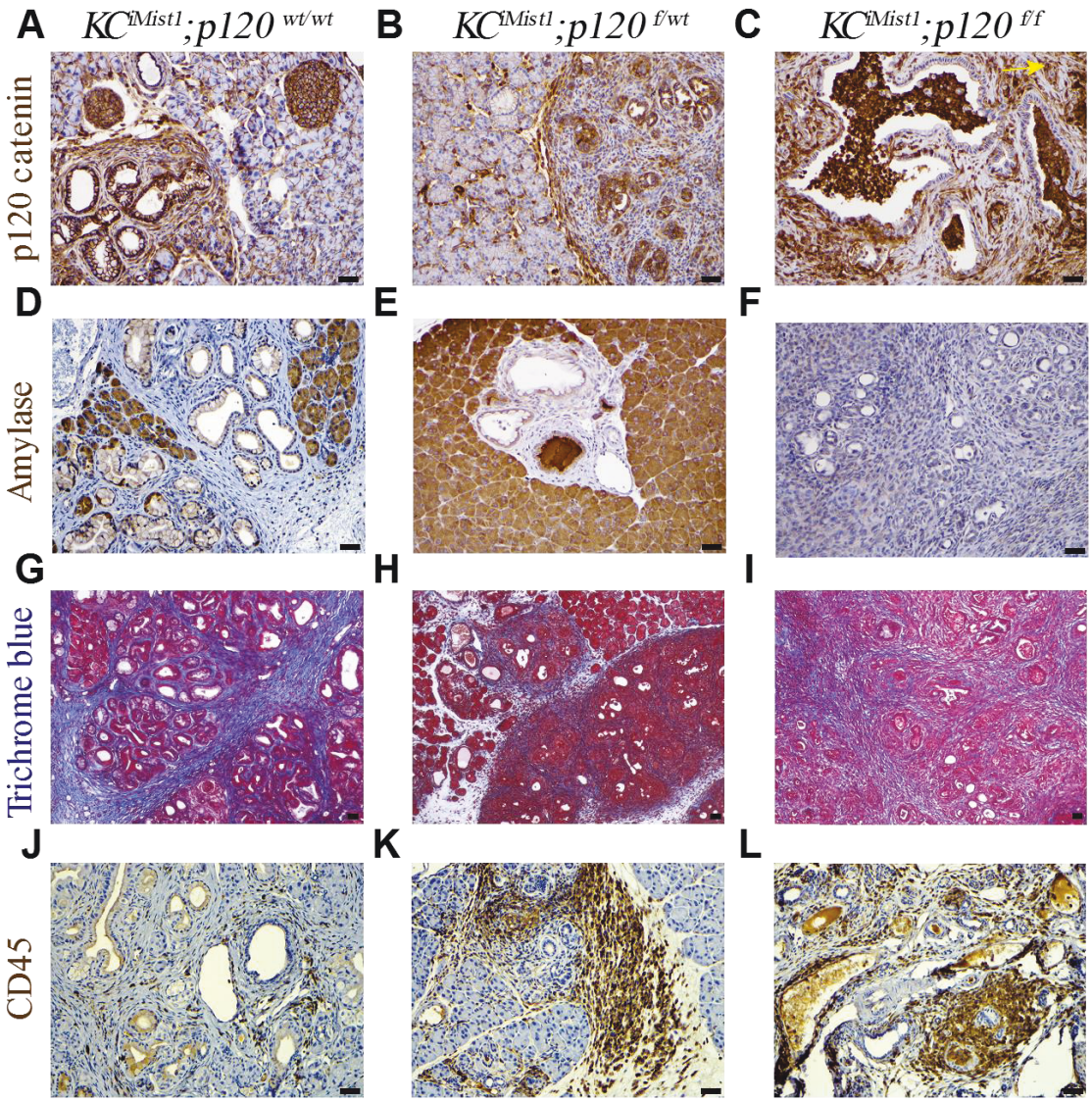

Figure 3.8. Pancreatic loss of p120 catenin cooperates with mutant Kras to form a unique cellular stroma. A,B) IHC for p120 catenin showed ubiquitous expression in *KC^{iMist1}; p120$^{wt/wt}$* and *KC^{iMist1}; p120$^{f/wt}$* pancreata. C) IHC showed loss of p120 catenin in *KC^{iMist1}; p120$^{f/f}$* pancreata with minimal mosaicism, which is highlighted with a yellow arrow. D-F) *KC^{iMist1}; p120$^{f/f}$* pancreata displayed reduction of amylase-expressing acinar cells when compared to *KC^{iMist1}; p120$^{f/wt}$* and *KC^{iMist1}; p120$^{wt/wt}$* pancreata. G-I) Trichrome blue staining showed a distinct cellular composition of fibrostroma in *KC^{iMist1}; p120$^{f/f}$* and *KC^{iMist1}; p120$^{f/wt}$* pancreata when compared to *KC^{iMist1}; p120$^{wt/wt}$* pancreata. J-L) *KC^{iMist1}; p120$^{f/f}$* and *KC^{iMist1}; p120$^{f/wt}$* pancreata displayed localized areas containing pronounced inflammation. Scale bars are 50μm.

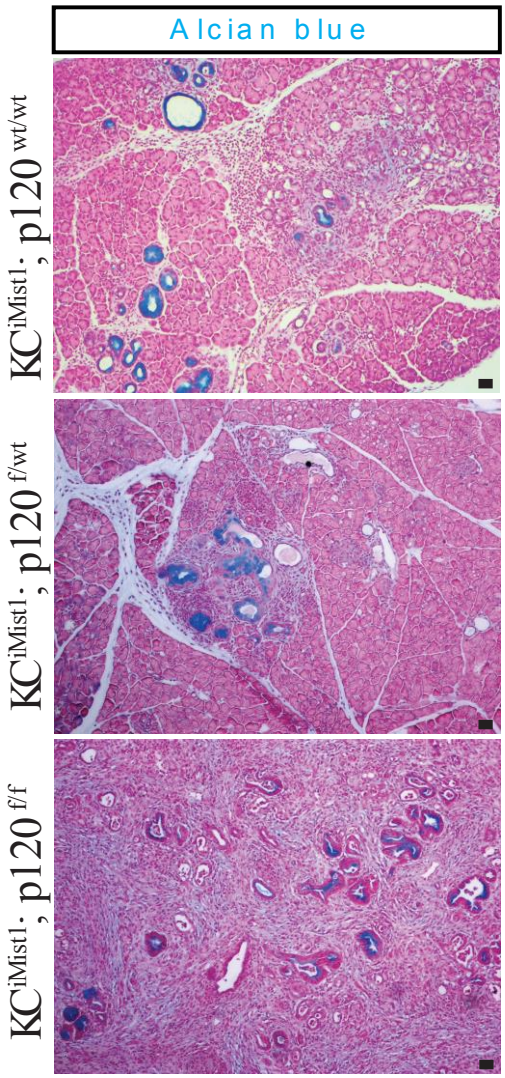

Figure 3.9. Alcian blue staining suggests PanIN formation in *KC^{iMist1}; p120$^{wt/wt}$*, *KC^{iMist1}; p120$^{f/wt}$*, and *KC^{iMist1}; p120$^{f/f}$* pancreata. Alcian blue staining showed the presence of PanIN lesions in *KC^{iMist1}; p120$^{wt/wt}$*, *KC^{iMist1}; p120$^{f/wt}$*, and *KC^{iMist1}; p120$^{f/f}$* pancreata. Scale bars are 50µm.

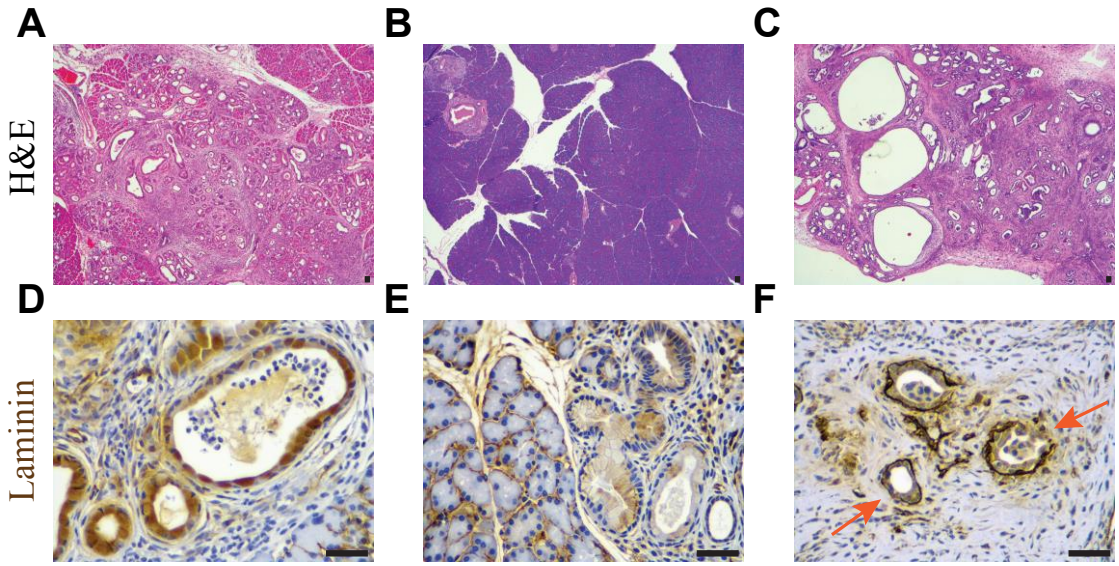

Figure 3.10. Basement membrane Laminin is disrupted in *KC^{iMist1}; p120$^{f/f}$* pancreata. A-C) *KC^{iMist1}; p120$^{f/f}$* pancreata displayed notable dilated ducts, which were not seen in *KC^{iMist1}; p120$^{wt/wt}$* and *KC^{iMist1}; p120$^{f/wt}$* pancreata. D-F) PanIN in *KC^{iMist1}; p120$^{wt/wt}$* and *KC^{iMist1}; p120$^{f/wt}$* pancreata showed very little basement membrane Laminin expression. Orange arrows point to regions in which contiguous basement membrane Laminin expression was disrupted in *KC^{iMist1}; p120$^{f/f}$* pancreata. Single cells expressing Laminin were also seen in the surrounding stroma. Scale bars are 50µm.

Lineage tracing reveals that loss of p120 catenin in cooperation with oncogenic Kras promotes striking cell extrusion

The exit of epithelial cells across the basement membrane of discernable epithelial structures, a process termed delamination, occurs in $KPC^{Pdx1}Y$ and $KC^{iMist1}Y$ PanIN mice and has been associated with EMT (Rhim et al., 2012). Since loss of p120 catenin in pancreatic cancer cell lines results in increased migration and invasion, also associated with EMT (Hamada et al., 2013), we next sought to determine if p120 catenin regulated delamination in PanIN mice. First, we examined expression of p120 catenin and E-cadherin in delaminated cells in 2 lineage-traced murine models of PanIN: $KPC^{Ptf1a}Y$ and $KC^{iMist1}G$ (Bailey et al., 2014; McAllister et al., 2014). Delaminated cells in $KPC^{Ptf1a}Y$ PanIN mice expressed decreased adherens junction proteins p120 catenin and E-cadherin when compared to their surrounding pancreatic epithelia (Figure 3.11A). $KC^{iMist1}G;$ $p120^{wt/wt}$ PanIN mice showed decreased p120 catenin and E-cadherin expression in non-epithelial delaminated cells, some of which have an elongated fibroblast cell morphology (Figure 3.11B). These data show that decreased expression of adherens junction proteins p120 catenin and E-cadherin is a manifest feature of delaminated cells in PanIN mice.

To further investigate the role of p120 catenin in delamination in Kras-induced premalignant pancreatic neoplasia, we next examined delaminated cells in lineage-traced $KC^{iMist1}G;$ $p120^{f/wt}$ and $KC^{iMist1}G;$ $p120^{f/f}$ mice. Monoallelic and biallelic loss of p120 catenin resulted in remarkable abundant delamination of GFP^+ cells that retained E-cadherin (Figure 3.11C,D). Both delamination, also termed basal epithelial cell extrusion (Y. Gu & Rosenblatt, 2012), as well as apical epithelial cell extrusion were significantly increased in $KC^{iMist1};$ $p120^{f/wt}$ and $KC^{iMist1};$ $p120^{f/f}$ pancreata when compared to $KC^{iMist1};$

p120$^{wt/wt}$ pancreata (Figure 3.12A-E). Abundant apical and basal epithelial cell extrusion was 100% penetrant in *KC*^{iMist1}*; p120*$^{f/wt}$ and *KC*^{iMist1}*; p120*$^{f/f}$ pancreata, with the phenotype evident in *KC*^{iMist1}*; p120*$^{f/f}$ pancreata by 2 weeks post tamoxifen injection (Figure 3.13A). Quantification of extruded isolated CK19^{+} cells revealed 838/7000 in *KC*^{iMist1}*; p120*$^{f/f}$ pancreata, 76/7000 in *KC*^{iMist1}*; p120*$^{f/wt}$ pancreata, and 19/7000 in *KC*^{iMist1}*; p120*$^{wt/wt}$ pancreata (Figure 3.13B). Lineage tracing in *KC*^{iMist1}*; p120*$^{f/f}$ pancreata showed extruded GFP^{+}, CK19^{+} single cells were negative for Vimentin, a marker of mesenchymal differentiation and EMT (Figure 3.13C). These data show that p120 catenin suppresses epithelial cell extrusion in Kras-driven pancreatic neoplasia. In addition, these data show that p120 catenin is not required for maintenance of either E-cadherin localization to cell membranes or epithelial cell identity after basal epithelial cell extrusion in the context of oncogenic Kras. Furthermore, basal epithelial cell extrusion resulting from biallelic p120 catenin loss is not associated with incomplete EMT.

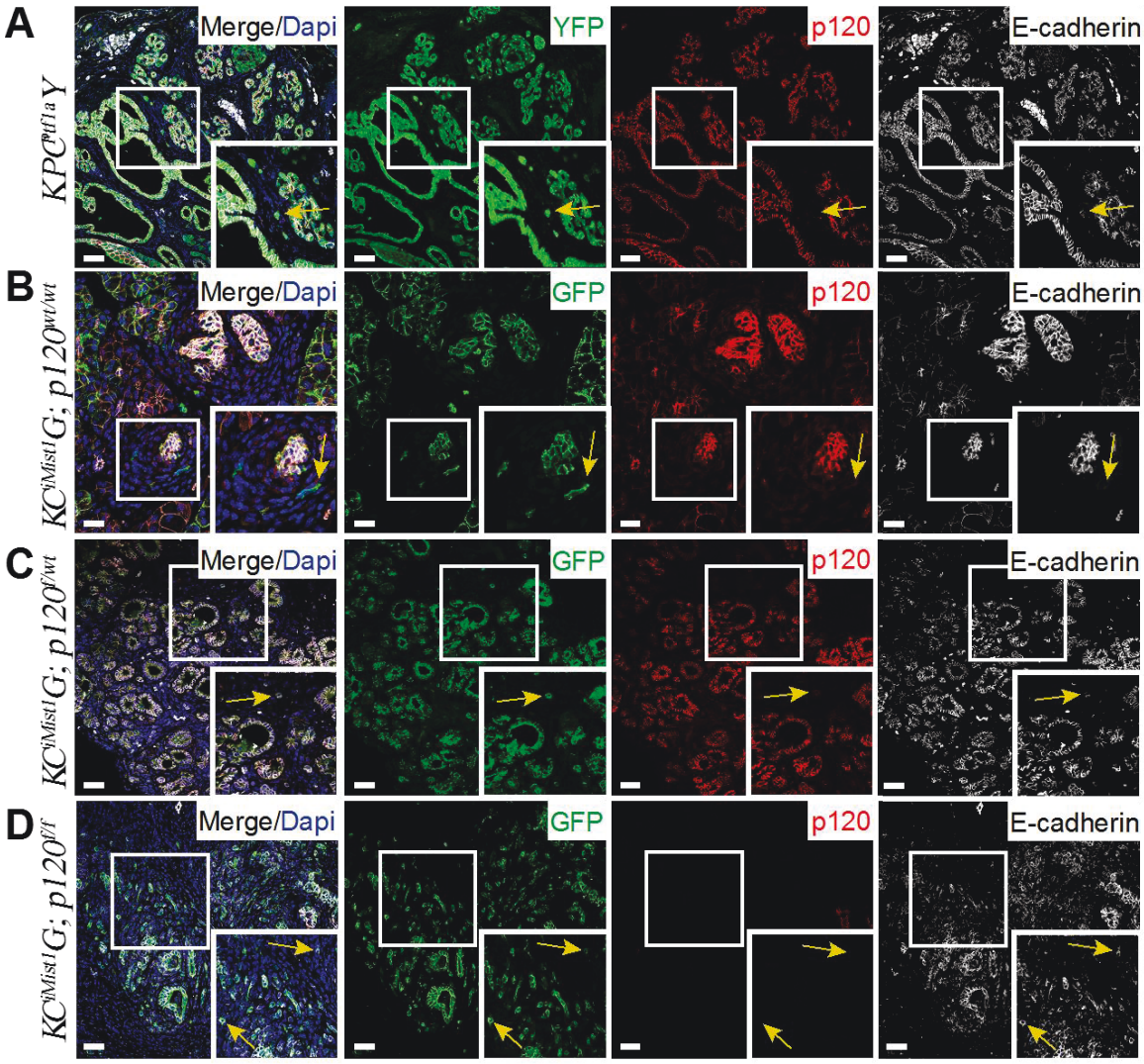

Figure 3.11. Loss of p120 catenin cooperates with oncogenic Kras to promote striking epithelial cell delamination. A,B) *KPC*^{Ptf1a}*Y* and *KC*^{iMist1}*G; p120*$^{wt/wt}$ mice show decreased expression of p120 catenin and E-cadherin in delaminated cells. Yellow arrows point to lineaged traced delaminated cells. C,D) Yellow arrows highlight abundant epithelial cell extrusion in *KC*^{iMist1}*G; p120*$^{f/wt}$ and *KC*^{iMist1}*G; p120*$^{f/f}$ mice.

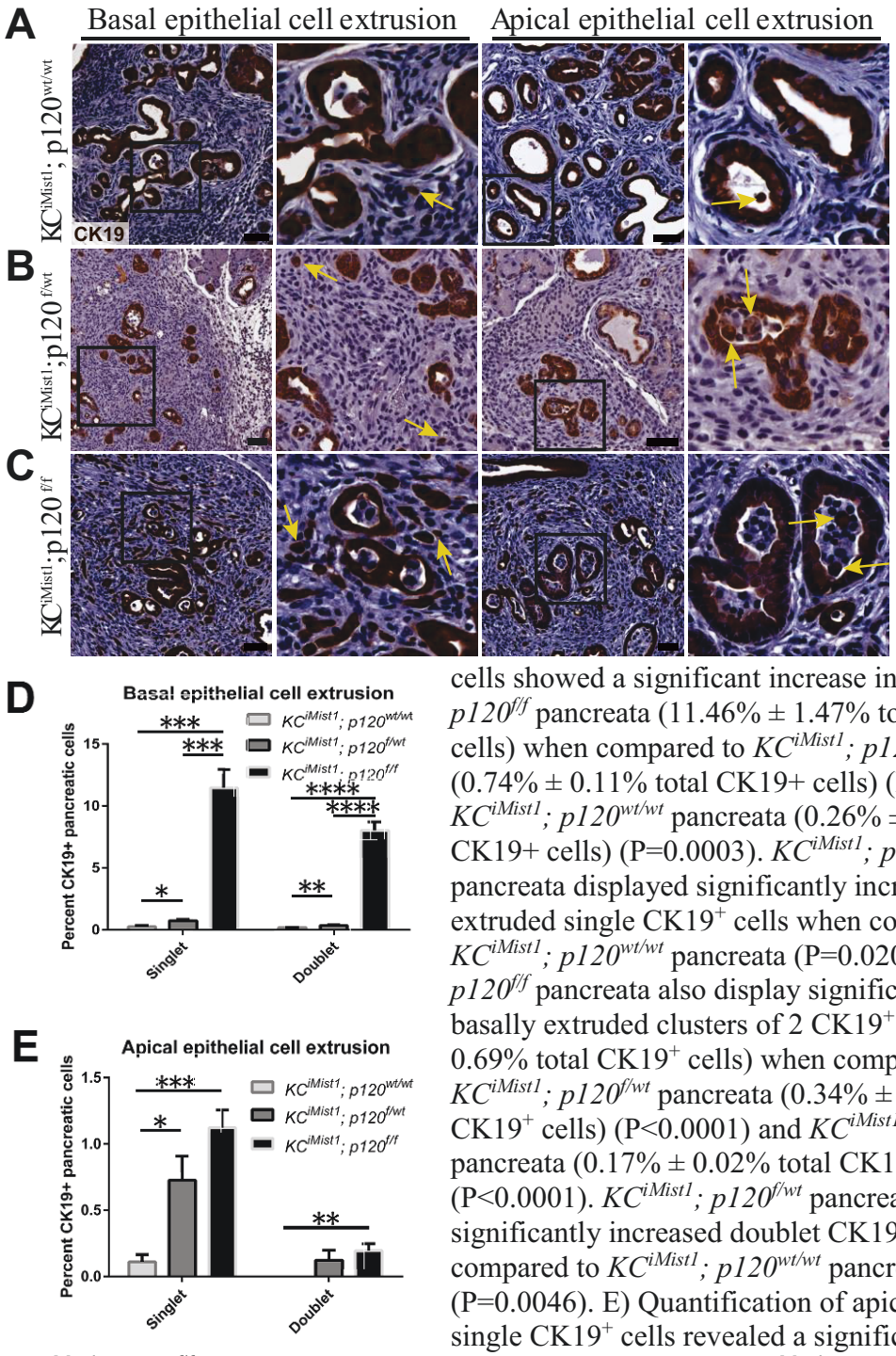

Figure 3.12. Loss of p120 catenin promotes epithelial cell extrusion in Kras-driven neoplasia. A-C) Yellow arrows show single apically and basally extruded CK19^{+} cells. D) Quantification of basally extruded CK19^{+} single cells showed a significant increase in *KC^{iMist1}; p120$^{f/f}$* pancreata (11.46% ± 1.47% total CK19^{+} cells) when compared to *KC^{iMist1}; p120$^{f/wt}$* pancreata (0.74% ± 0.11% total CK19+ cells) (P=0.0003) and *KC^{iMist1}; p120$^{wt/wt}$* pancreata (0.26% ± 0.12% total CK19+ cells) (P=0.0003). *KC^{iMist1}; p120$^{f/wt}$* pancreata displayed significantly increased basally extruded single CK19^{+} cells when compared to *KC^{iMist1}; p120$^{wt/wt}$* pancreata (P=0.0208). *KC^{iMist1}; p120$^{f/f}$* pancreata also display significantly increased basally extruded clusters of 2 CK19^{+} cells (8.03% ± 0.69% total CK19^{+} cells) when compared to *KC^{iMist1}; p120$^{f/wt}$* pancreata (0.34% ± 0.05% total CK19^{+} cells) (P<0.0001) and *KC^{iMist1}; p120$^{wt/wt}$* pancreata (0.17% ± 0.02% total CK19^{+} cells) (P<0.0001). *KC^{iMist1}; p120$^{f/wt}$* pancreata also show significantly increased doublet CK19^{+} cells when compared to *KC^{iMist1}; p120$^{wt/wt}$* pancreata (P=0.0046). E) Quantification of apically extruded single CK19^{+} cells revealed a significant increase in *KC^{iMist1}; p120$^{f/f}$* pancreata (1.12% ± 0.13%) (P=0.0004) and *KC^{iMist1}; p120$^{f/wt}$* pancreata (0.73% ± 0.18%) (P=0.018) when compared to *KC^{iMist1}; p120$^{wt/wt}$* pancreata (0.11% ± 0.06%). *KC^{iMist1}; p120$^{f/f}$* pancreata also showed significantly increased apically extruded CK19^{+} doublets when compared to *KC^{iMist1}; p120$^{wt/wt}$* pancreata (P=0.0093). Scale bars are 50µm.

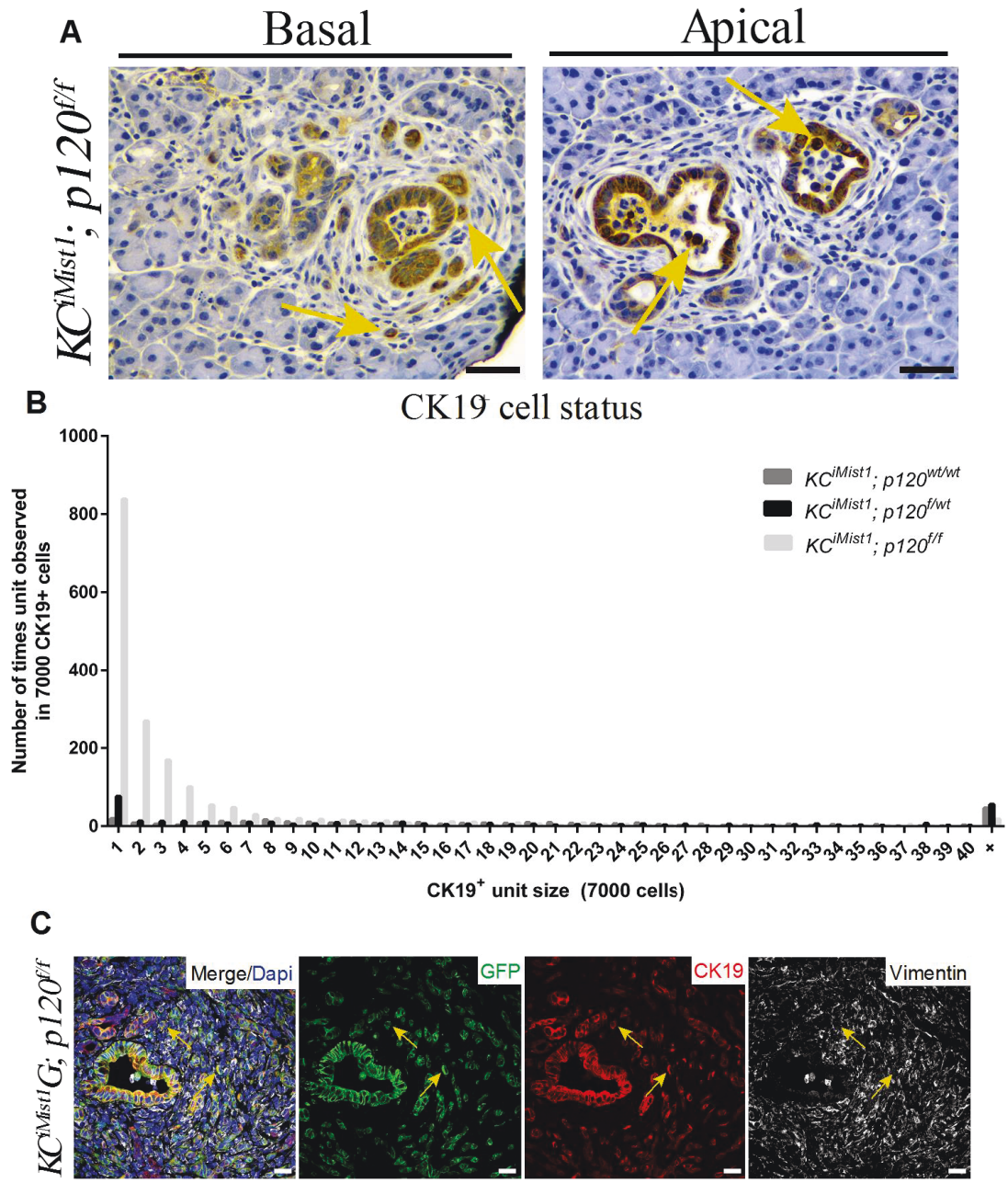

Figure 3.13. Characterization of epithelial cell extrusion in *KC^{iMist1}; p120$^{wt/wt}$*, *KC^{iMist1}; p120$^{f/wt}$*, and *KC^{iMist1}; p120$^{f/f}$* pancreata. A) *KC^{iMist1}; p120$^{f/f}$* pancreata display abundant apical and basal epithelial cell extrusion 2 weeks post tamoxifen injection. Yellow arrows point to extruded CK19^{+} single cells. B) Quantification of CK19^{+} unit size unbiased to direction of extrusion is shown for *KC^{iMist1}; p120$^{wt/wt}$*, *KC^{iMist1}; p120$^{f/wt}$*, and *KC^{iMist1}; p120$^{f/f}$* pancreata (n=7000 cells in 4 animals for each genotype). C) IF for GFP, CK19, and Vimentin showed that basally extruded single cells in *KC^{iMist1}; p120$^{f/f}$* pancreata were Vimentin negative. Yellow arrows point to single GFP^{+}, CK19^{+}, Vimentin^{-} basally extruded cells. Scale bars are 50μm.

p120 catenin loss in the context of cerulean-induced pancreatitis promotes epithelial cell extrusion

Biallelic loss of p120 catenin during pancreas development promotes apical epithelial cell extrusion (Hendley et al., 2015). Luminal cell extrusion has also been reported with loss of p120 catenin in developing kidney and MDCK cysts (Marciano et al., 2011). Given the abundant basal and apical epithelial cell extrusion observed with p120 catenin loss in cooperation with oncogenic Kras in premalignant pancreatic neoplasia, we next sought to interrogate the extrusion behavior of adult pancreatic cells in regenerating pancreas. To this end, acute pancreatitis was induced in $C^{iMist1}; p120^{wt/wt}$, $C^{iMist1}; p120^{f/wt}$, and $C^{iMist1}; p120^{f/f}$ mice (Figure 3.14A). $C^{iMist1}; p120^{f/f}$ pancreata showed significantly increased susceptibility to injury when compared to $C^{iMist1}; p120^{wt/wt}$ and $C^{iMist1}; p120^{f/wt}$ pancreata (Figures 3.16 and 3.17). Quantification of apical epithelial cell extrusion revealed a significant increase in $C^{iMist1}; p120^{f/f}$ pancreata when compared to $C^{iMist1}; p120^{wt/wt}$ and $C^{iMist1}; p120^{f/wt}$ pancreata (Figure 3.14B,C). In addition, $C^{iMist1}; p120^{f/f}$ and $C^{iMist1}; p120^{f/wt}$ pancreata showed significantly increased basal epithelial cell extrusion when compared to $C^{iMist1}; p120^{wt/wt}$ pancreata (Figure 3.15A,B). These data suggest that p120 catenin regulates both apical and basal epithelial cell extrusion in adult mouse pancreas in the context of acute pancreatitis.

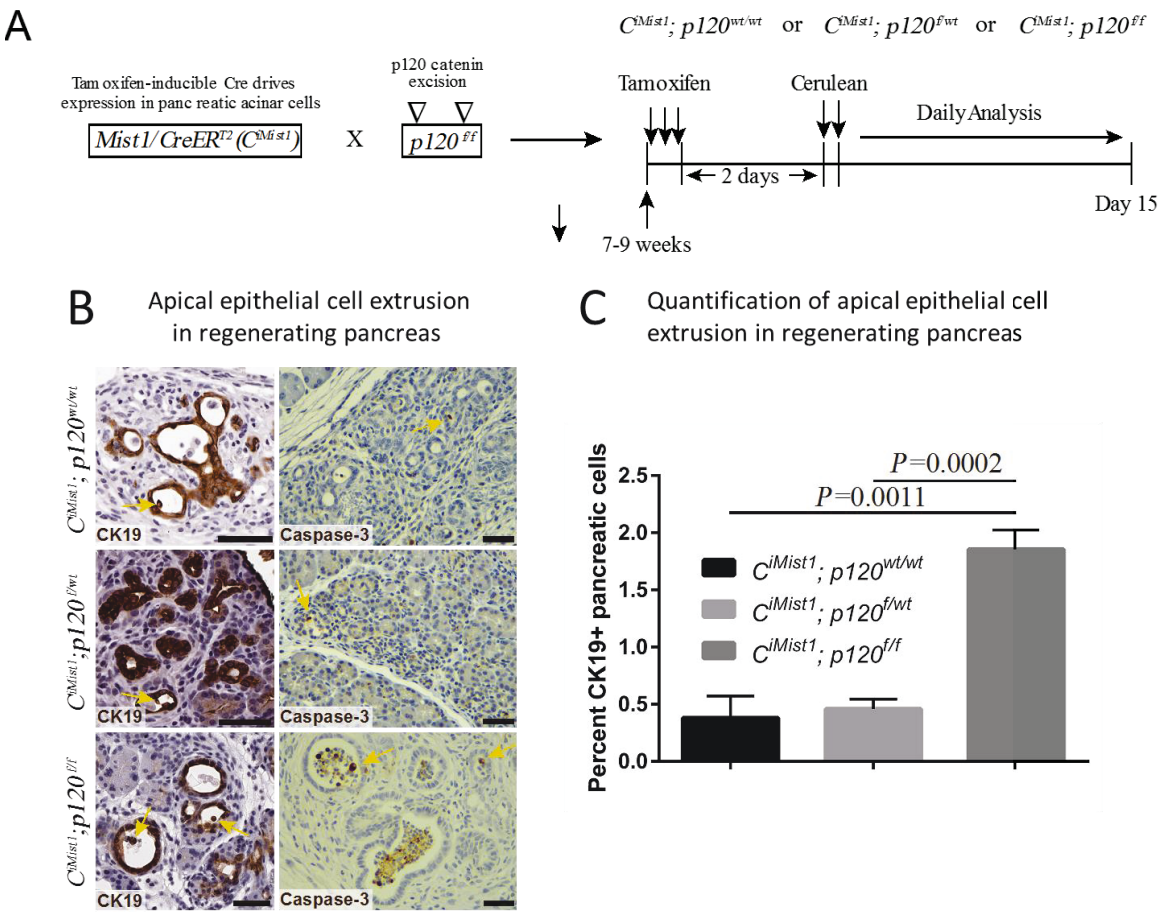

Figure 3.14. p120 catenin regulates apical epithelial cell extrusion in cerulean-induced acute pancreatitis. A) Schematic for tamoxifen injection and induction of experimental acute pancreatitis. B) CK19 IHC showed apically extruded epithelial cells in lumens of metaplastic duct lesions. A subset of apically extruded cells are positive for cleaved Caspase-3. Yellow arrows point to apically extruded cells. C) Quantification of apically extruded CK19^{+} cells at Days 5 and 7 post cerulean administration revealed a significant increase in *C^{iMist1}; p120$^{f/f}$* pancreata (1.86% ± 0.17%, n=8750 cells in 6 pancreata) when compared to *C^{iMist1}; p120$^{f/wt}$* pancreata (0.46% ± 0.08% , n=3323 cells in 4 pancreata) and *C^{iMist1}; p120$^{wt/wt}$* pancreata (0.38% ± 0.19% , n=3288 cells in 3 pancreata). Scale bars are 50µm.

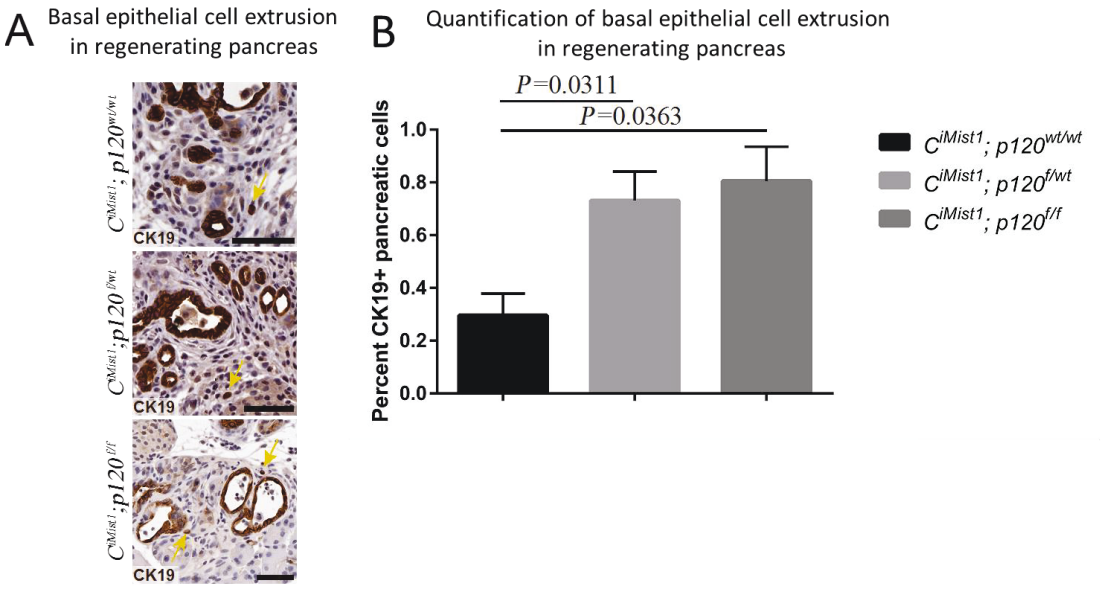

Figure 3.15. p120 catenin regulates basal epithelial cell extrusion in cerulean-induced acute pancreatitis. A) CK19 IHC showed basally extruded epithelial cells in stroma surrounding metaplastic duct lesions. B) Quantification of basally extruded CK19^{+} cells at Days 5 and 7 post cerulean treatment showed a significant increase in C^{iMist1}; $p120^{f/f}$ pancreata (0.81% ± 0.13%, n=10710 cells in 6 pancreata) and C^{iMist1}; $p120^{f/wt}$ pancreata (0.73% ± 0.11%, n=4594 cells in 4 pancreata) when compared to C^{iMist1}; $p120^{wt/wt}$ pancreata (0.30% ± 0.08%, n=6217 cells in 3 pancreata). Scale bars are 50μm.

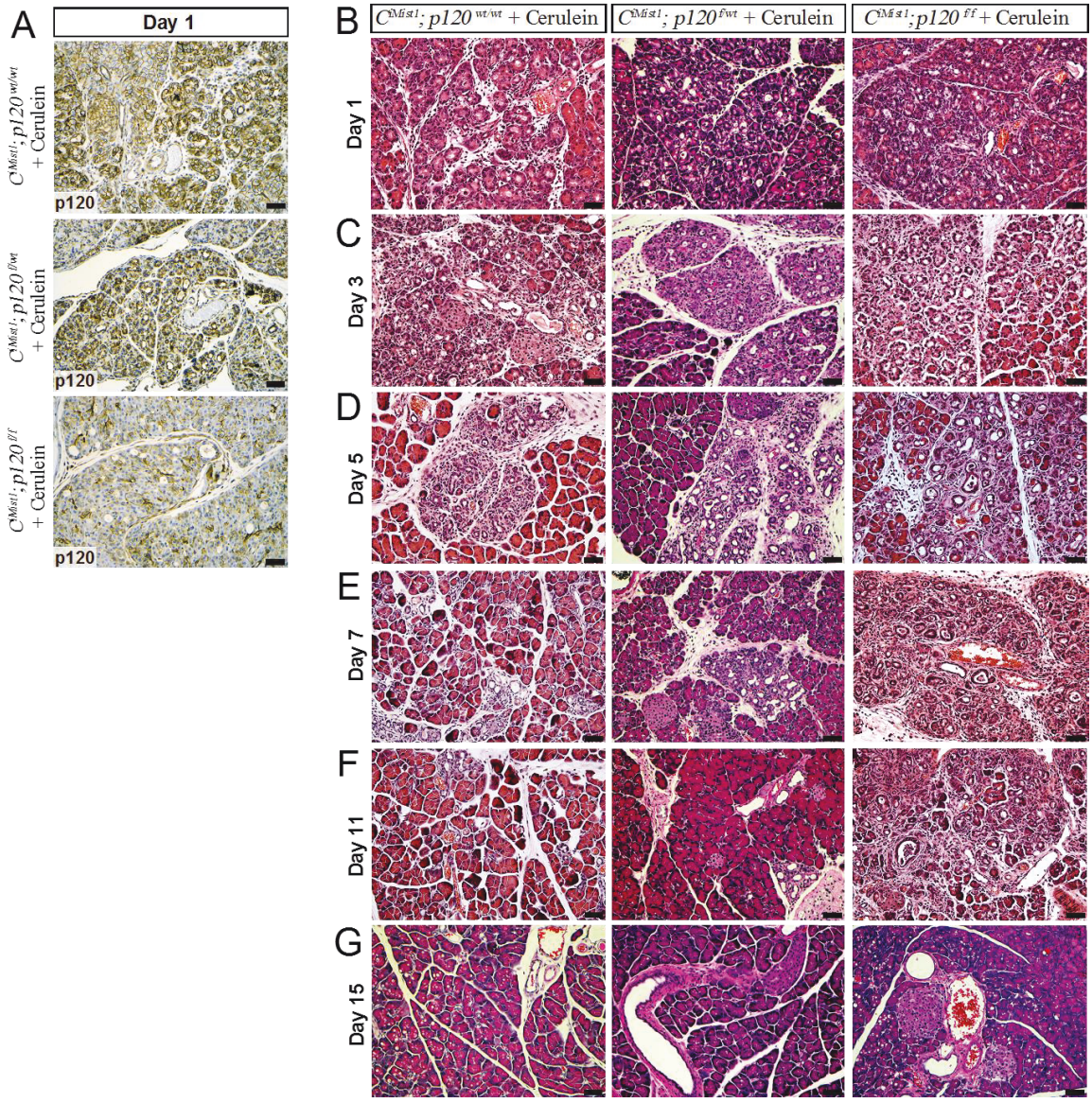

Figure 3.16. p120 catenin loss leads to increased susceptibility to pancreatic injury. A) IHC showed p120 catenin loss in *C^{iMist1}; p120$^{f/f}$* pancreata. B-G) Representative images from a time course show a delayed capacity for *C^{iMist1}; p120$^{f/f}$* pancreata to regenerate following cerulean-induced pancreatic injury. Scale bars are 50µm.

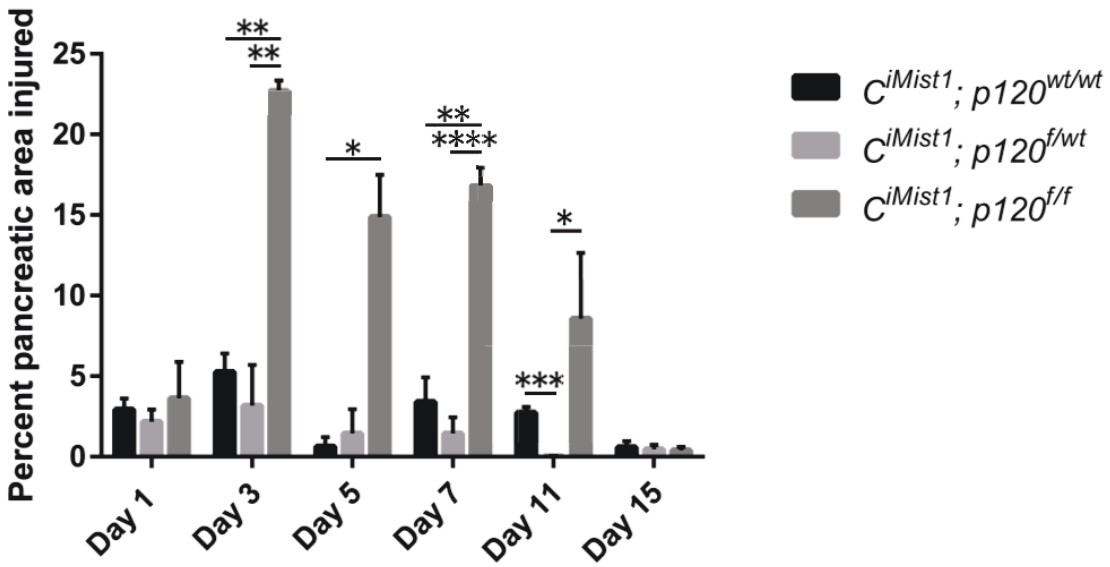

Figure 3.17. *C^{iMist1}; p120$^{f/f}$* pancreata show significantly delayed regeneration in the context of cerulean-induced pancreatitis. On Day 1 post cerulean treatment, no significant difference in injury was observed between *C^{iMist1}; p120$^{f/f}$* (n=3 pancreata), *C^{iMist1}; p120$^{f/wt}$* (n=3 pancreata), and *C^{iMist1}; p120$^{wt/wt}$* (n=2 pancreata) mice. Three days post cerulean administration, *C^{iMist1}; p120$^{f/f}$* mice (n=2 pancreata) showed significantly increased injury when compared to *C^{iMist1}; p120$^{f/wt}$* (n=4 pancreata, P=0.007) and *C^{iMist1}; p120$^{wt/wt}$* (n=2 pancreata, P=0.0063) mice. Five days after cerulean treatment, *C^{iMist1}; p120$^{f/f}$* mice (n=3 pancreata) showed significantly increased injury when compared to *C^{iMist1}; p120$^{wt/wt}$* (n=2 pancreata, P=0.0249) mice and nearly significantly increased injury when compared to *C^{iMist1}; p120$^{f/wt}$* (n=2 pancreata, P=0.0541) mice. On day 7 post cerulean administration, *C^{iMist1}; p120$^{f/f}$* mice (n=3 pancreata) continued to display significantly increased injury when compared to *C^{iMist1}; p120$^{f/wt}$* (n=5 pancreata, P=<0.0001) and *C^{iMist1}; p120$^{wt/wt}$* (n=2 pancreata, P=0.0054) mice. Eleven days after cerulean treatment, *C^{iMist1}; p120$^{f/f}$* mice (n=2 pancreata) showed significantly increased injury when compared to *C^{iMist1}; p120$^{f/wt}$* (n=4 pancreata, P=0.0271) mice. On day 15 post cerulean treatment, *C^{iMist1}; p120$^{f/f}$* (n=4 pancreata), *C^{iMist1}; p120$^{f/wt}$* (n=7 pancreata), and *C^{iMist1}; p120$^{wt/wt}$* (n=2 pancreata) pancreata displayed no significant differences in injury and were nearly completely regenerated.

Epithelial cells that extrude basally in KC^{iMist1}; $p120^{wt/wt}$, KC^{iMist1}; $p120^{f/wt}$, and KC^{iMist1}; $p120^{f/f}$ pancreata survive

We next sought to determine the fate of both apically and basally extruded epithelial cells in KC^{iMist1}; $p120^{wt/wt}$, KC^{iMist1}; $p120^{f/wt}$, and KC^{iMist1}; $p120^{f/f}$ pancreata. Since epithelial cells can extrude apically by a mechanism involving S1P/S1pr2 signaling and activation of cleaved Caspase-3 *(Y. Gu et al., 2011; Rosenblatt et al., 2001)*, we next examined cleaved Caspase-3 expression in extruded $CK19^+$ cells. Consistent with this previously reported mechanism of apical cell extrusion, we observed cleaved Caspase-3 expression in apically extruded $CK19^+$ cells in KC^{iMist1}; $p120^{wt/wt}$, KC^{iMist1}; $p120^{f/wt}$, and KC^{iMist1}; $p120^{f/f}$ pancreata (Figure 3.18A-C). However, we observed no cleaved Caspase-3 expression in isolated, basally extruded $CK19^+$ cells, suggesting that basally extruded $CK19^+$ cells exit intact pancreatic epithelial structures and remain viable (Figure 3.18A-C).

Basally extruded isolated epithelial cells in KC^{iMist1}; $p120^{f/f}$ pancreata display aneuploidy and nuclear enlargement

Because we observed prominent nucleoli and nuclear enlargement of basally extruded epithelial cells in KC^{iMist1}; $p120^{f/f}$ pancreata, we next analyzed the DNA content of isolated $CK19^+$ cells in KC^{iMist1}; $p120^{f/f}$ pancreata using OTMIAS Image Analysis Software on Feulgen stained slides (Figure 3.19A-C). A population of pancreatic cells was observed with a DNA index of 1.5, which was indicative of aneuploidy (Figure 3.19C). In addition, 8.3% (21/253) pancreatic cells analyzed showed abnormal DNA content with a DNA index of ≥ 2.5 (Figure 3.19C). The histology of KC^{iMist1}; $p120^{f/f}$

pancreata thus comprises a very unique phenotype with overall benign neoplasia and reactive stroma containing isolated epithelial cells that display features of malignancy including enlarged, hyperchromatic and pleomorphic nuclei, prominent nucleoli, aneuploidy and occasional binuclear cells. These findings suggest that p120 catenin loss in the context of oncogenic Kras promotes formation of invasive pancreatic neoplasia (Figure 3.19D).

Isolated epithelial cells in human PDAC misexpress p120 catenin

As we identified that loss of p120 catenin in cooperation with oncogenic Kras promotes invasion of epithelial cells displaying characteristics of malignancy in pancreatic neoplasia, we next examined expression of p120 catenin in isolated epithelial cells in human PDAC. Single malignant epithelial cells in human PDAC are depicted in Figure 3.20A,B. Quantification of p120 catenin subcellular localization in 253 isolated epithelial cells from 17 patients with PDAC showed a sparse 4.74% of cells with normal membrane labeling and 95.26% of cells with predominant cytoplasmic or absent p120 catenin localization (Figure 3.20C,D). These data show that altered p120 catenin subcellular localization is a distinctive feature of isolated malignant epithelial cells in human PDAC.

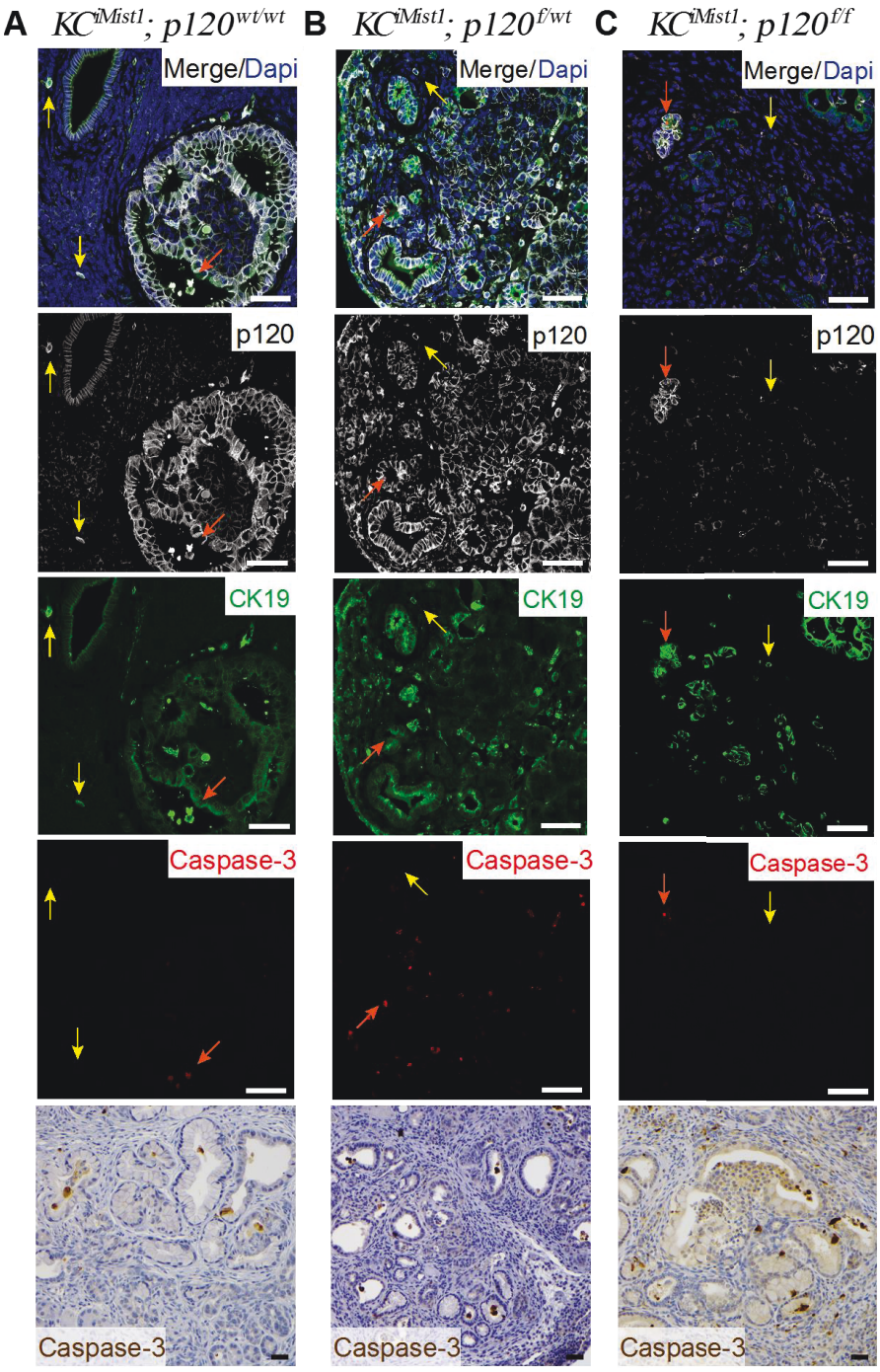

Figure 3.18. CK19$^+$ cells that exit pancreatic epithelial structures basally survive. A-C) Immunolabeling showed CK19$^+$ cells that extrude apically express cleaved Caspase-3, and CK19$^+$ cells that extrude basally lack cleaved Caspase-3 labeling in *KC^{iMist1}; p120$^{wt/wt}$*, *KC^{iMist1}; p120$^{f/wt}$*, and *KC^{iMist1}; p120$^{f/f}$* pancreata. Yellow arrows point to basally extruded CK19$^+$ cells, and orange arrows show apically extruded CK19$^+$ cells. Scale bars are 50μm.

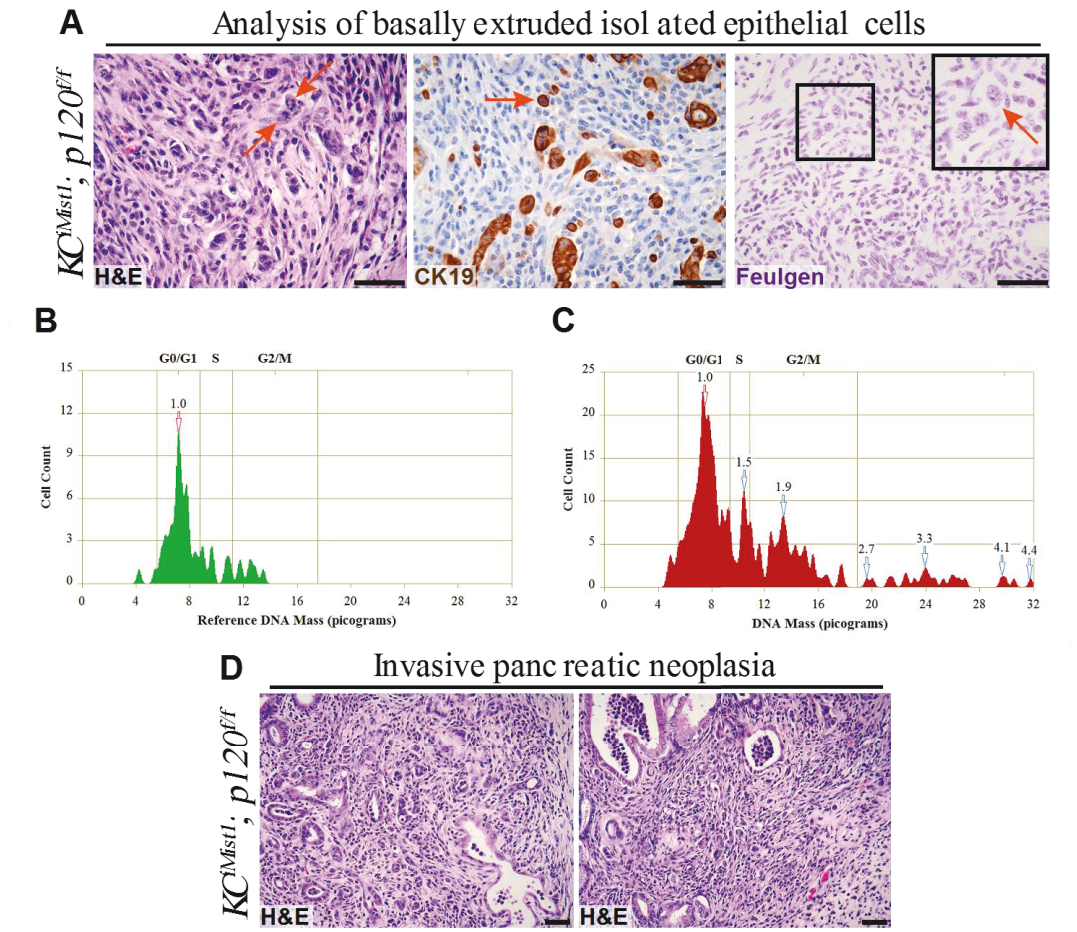

Figure 3.19. Basally extruded epithelial cells in *KC^{iMist1}; p120$^{f/f}$* pancreata display characteristics of malignancy. A) Orange arrows in the H&E stained tissue section point to atypical cells displaying nuclear enlargement in a *KC^{iMist1}; p120$^{f/f}$* pancreas. The orange arrow in the CK19 IHC image shows a cell with abnormal DNA content in a *KC^{iMist1}; p120$^{f/f}$* pancreas. The orange arrow in the Feulgan stained image points to an example of an isolated epithelial cell analyzed for DNA content. B,C) The green histogram represents DNA ploidy analysis on gastric epithelial cells (diploid control), and the red histogram shows DNA ploidy analysis on isolated basally extruded CK19^{+} pancreatic cells. 53 control diploid cells and 253 basally extruded cells were analyzed. The first peak represents cells in the G_0/G_1 phase of the cell cycle, which have a DNA index of 1.0 and DNA content of 2C (2N or diploid). Cells in the G_2/M phase have a DNA index of 2.0 and DNA content of 4C (4N or tetraploid). Cells with greater than 5C (DNA index 2.5) in the red histogram have abnormal DNA content. There are no control diploid cells with >5C (green histogram). The peak with DNA index 1.5 in the red histogram represents an aneuploid peak. D) H&E images show histology of *KC^{iMist1}; p120$^{f/f}$* pancreata. Scale bars are 50µm.

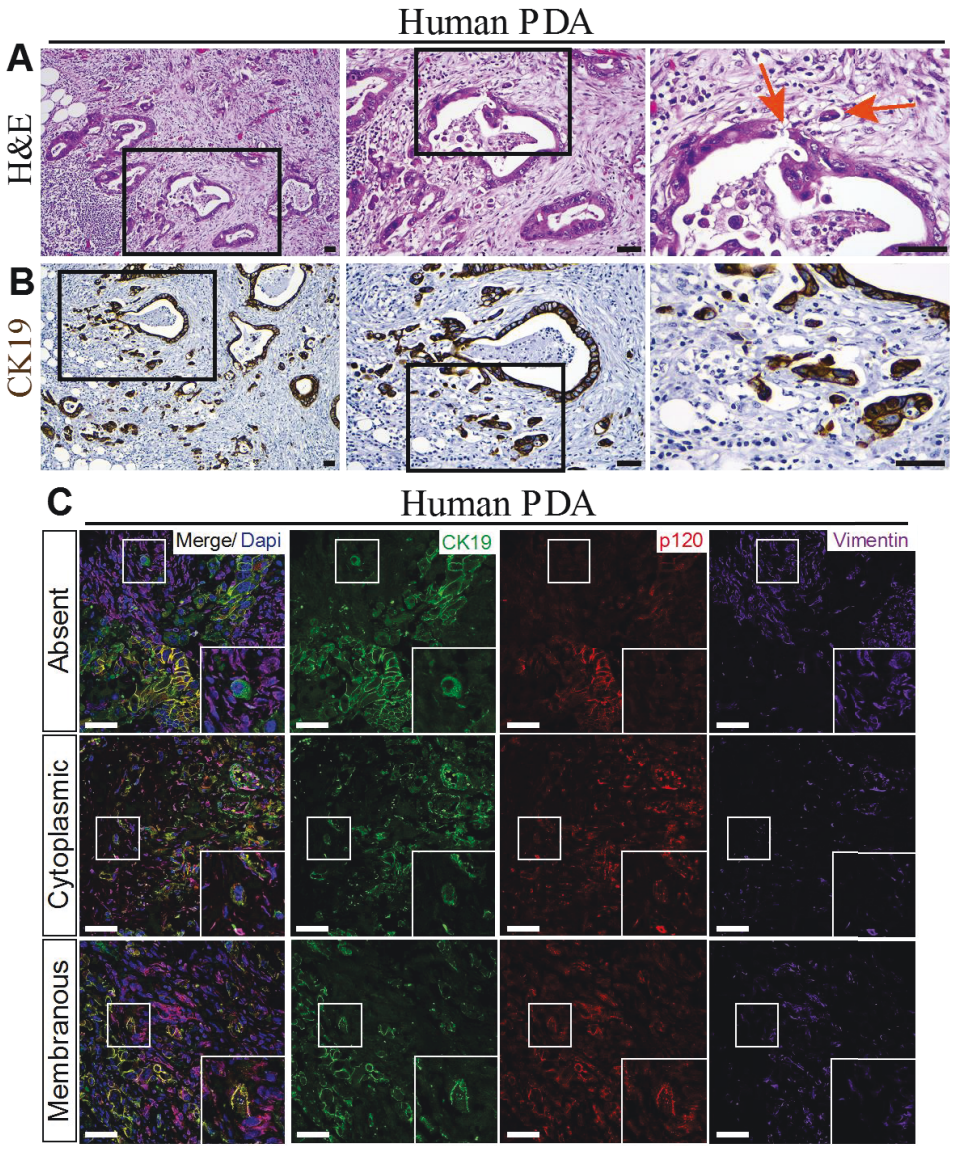

Figure 3.20. p120 catenin expression in isolated epithelial cells in human PDAC. A) H&E staining of human PDAC shows an isolated epithelial cell, highlighted with an orange arrow (right), and discontinuous epithelium in a lesion, also highlighted by an orange arrow (left). B) CK19 IHC shows isolated epithelial cells in human PDAC. C) IF images used to score p120 catenin subcellular localization in isolated $CK19^{+}$ $Vimentin^{-}$ cells show absent, cytoplasmic, and membranous p120 catenin labeling. D) An analysis of isolated epithelial cells in human pancreatic cancer defined as $CK19^{+}$ $Vimentin^{-}$ showed 4.74% (12/253) of cells with predominant membranous p120 catenin, 50.20% (127/253) of cells with predominant cytoplasmic p120 catenin, and 45.06% (114/253) of cells with predominant absent p120 catenin. Scale bars are 50µm.

KC^{iMist1}; $p120^{f/f}$ pancreata display a unique transcriptome signature and a downregulated S1P biosynthetic pathway

As a means to identify the mechanisms by which p120 catenin ablation in cooperation with oncogenic Kras promotes epithelial cell extrusion, we performed whole transcriptome analysis on fluorescence activated cell sorted (FACS) GFP^+ pancreatic cells in $KC^{iMist1}G; p120^{wt/wt}$ and $KC^{iMist1}G; p120^{f/f}$ mice 2 weeks post tamoxifen injection (Figure 3.21A). IPA analysis showed 56 statistically significant differentially expressed pathways, several of which are related to actin cytoskeleton signaling, the inflammatory response, and cell adhesion and migration (Figure 3.21B). Cytoplasmic p120 catenin regulates Rho family GTPases by inhibiting RhoA and activating Rac1 and Cdc42, which together modulate cytoskeleton organization and cell migration (Noren et al., 2000). Previously, we showed that actin cytoskeleton organization was disrupted in $C^{Pdx1}; p120^{f/f}$ pancreata, and that this observation was associated with increased cytoplasmic PKCζ, a known modulator of actin cytoskeleton dynamics (Hendley et al., 2015). Here, we similarly find that $KC^{iMist1}; p120^{f/f}$ pancreata show increased cytoplasmic PKCζ when compared to $KC^{iMist1}; p120^{f/wt}$ and $KC^{iMist1}; p120^{wt/wt}$ pancreata (Figure 3.22A-C). Expression of adherens junction components E-cadherin and β-catenin was also reduced in $KC^{iMist1}; p120^{f/f}$ pancreata (Figure 3.22D-I). In addition, IHC confirmed intrinsic activation of NF-kB in $KC^{iMist1}; p120^{f/f}$ pancreata (Figure 3.22J-L).

Since defective S1P/S1pr2 mediated cell extrusion in the context of mutant Kras has been shown to shift the predominant direction of cell extrusion from apical to basal (G. Slattum et al., 2014), we next queried whether p120 catenin regulated S1P/S1pr2 signaling. We performed qPCR on FACS-sorted GFP^+ pancreatic cells in $KC^{iMist1}G;$

p120$^{wt/wt}$ and *KC*^{iMist1}*G; p120*$^{f/f}$ mice at 1 month post tamoxifen injection (Figure 3.23A). We found an overall decrease in expression of genes mediating S1P/S1pr2 signaling (Figure 3.23A). The expression of genes involved in the biosynthetic pathway of S1P, *Sphk1* and *Sphk2,* was significantly decreased -5.83 fold and -3.96 fold, respectively, suggesting that p120 catenin regulates biosynthesis of S1P in a mutant Kras-dependent context. We next investigated the relationship between p120 catenin loss and epithelial cell extrusion mediated by S1P/S1pr2 signaling using an epithelial, Kras mutant, human pancreatic cancer cell line. CFPAC-1 cells express both p120 catenin and S1pr2 and form spheres (CFPAC-1 spheres) that can extrude cells basally when grown in Matrigel (Figure 3.23B,C). The percentage of CFPAC-1 spheres extruding epithelial cells basally increased significantly following p120 catenin knockdown (Figure 3.23D). Furthermore, the specific S1pr2 agonist CYM-5520 (Satsu et al., 2013) significantly decreased the frequency of basal extrusion in p120 catenin-deficient spheres to levels similar to those observed in p120 catenin-expressing spheres (Figure 3.23D). No significant difference was observed between extruded live and dead single cells in any treatment condition (data not shown). The ability of restored S1P/S1pr2 signaling to rescue the p120 catenin loss of function phenotype indicates a direct mechanism by which p120 catenin loss promotes increased basal epithelial cell extrusion.

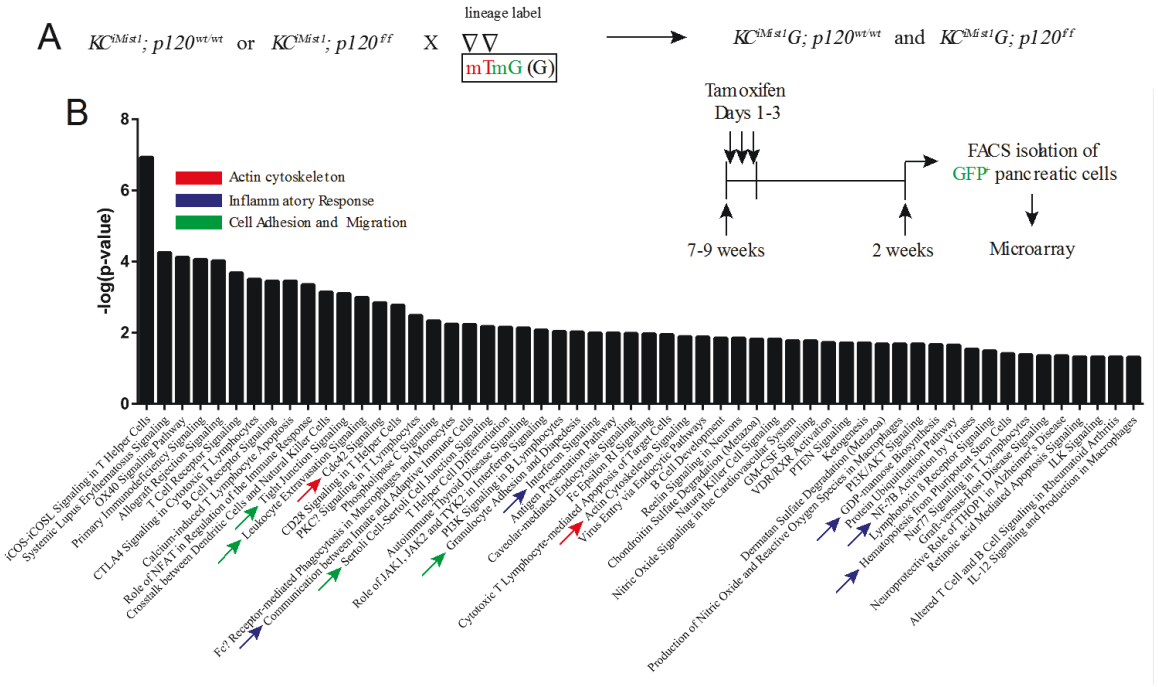

Figure 3.21. Loss of p120 catenin induces significant transcriptional changes in genes affecting actin cytoskeleton arrangement, cell adhesion and migration, and the inflammatory response. A) Sorting strategy for microarray in *KC^{iMist1}; p120$^{wt/wt}$* and *KC^{iMist1}; p120$^{f/f}$* mice. B) IPA analysis showed significant differentially expressed pathways including pathways involved in organization of actin cytoskeleton (red arrows), inflammatory response (blue arrows), and cell adhesion and migration (green arrows).

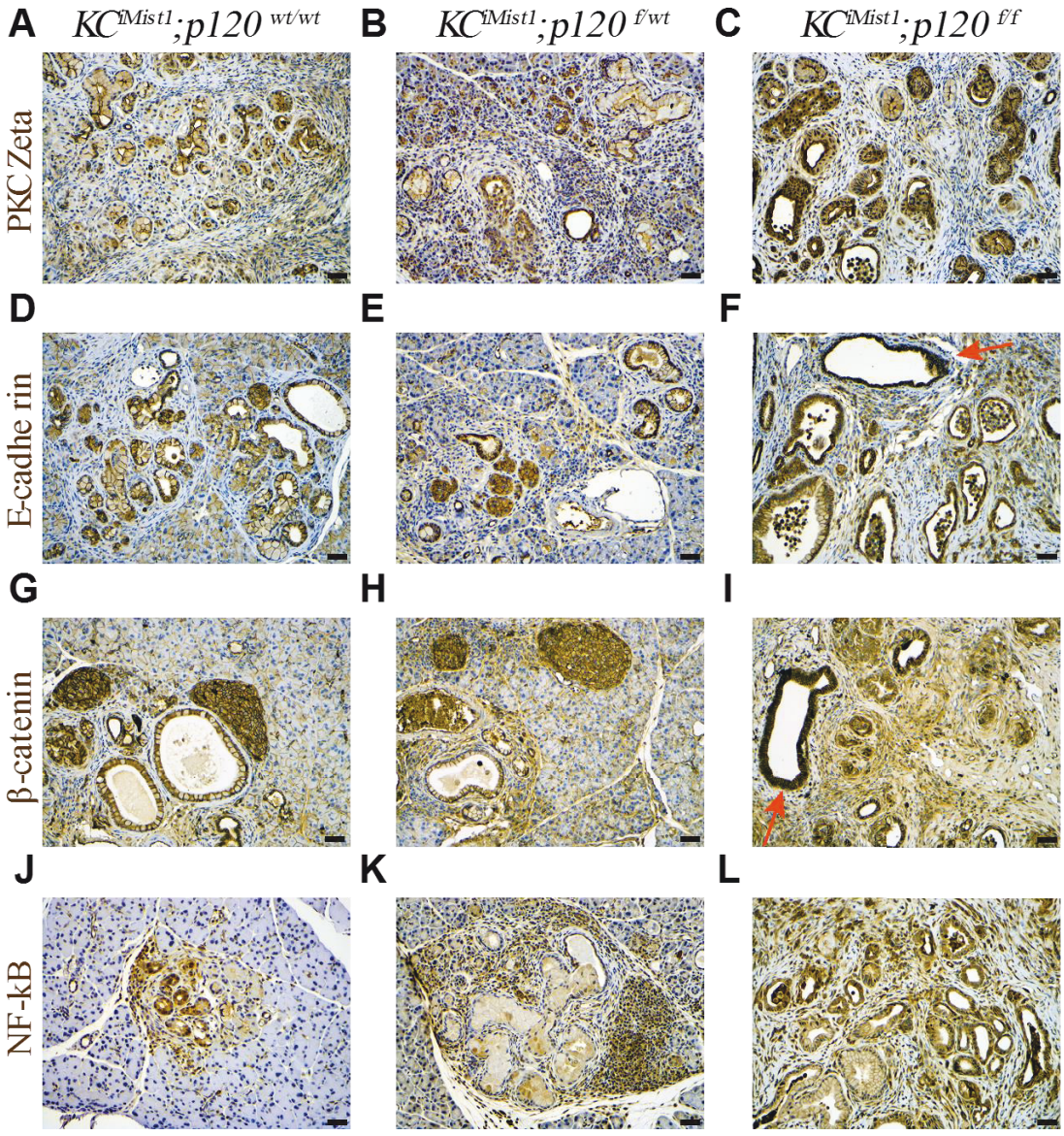

Figure 3.22. Loss of p120 catenin results in decreased adherens junction proteins and disruption of cytoskeletal organization. A-C) KC^{iMist1}; $p120^{f/f}$ pancreata showed increased cytoplasmic localization of PKCζ when compared to KC^{iMist1}; $p120^{f/wt}$ pancreata and KC^{iMist1}; $p120^{wt/wt}$ pancreata. D-I) Adherens junction members E-cadherin and β-catenin were reduced in KC^{iMist1}; $p120^{f/f}$ pancreata when compared to KC^{iMist1}; $p120^{f/wt}$ pancreata and KC^{iMist1}; $p120^{wt/wt}$ pancreata. Orange arrows point to ductal epithelium that expresses p120 catenin. J-L) KC^{iMist1}; $p120^{f/f}$ pancreata showed substantial activation of NF-kB in both epithelial and stromal compartments. Scale bars are 50μm.

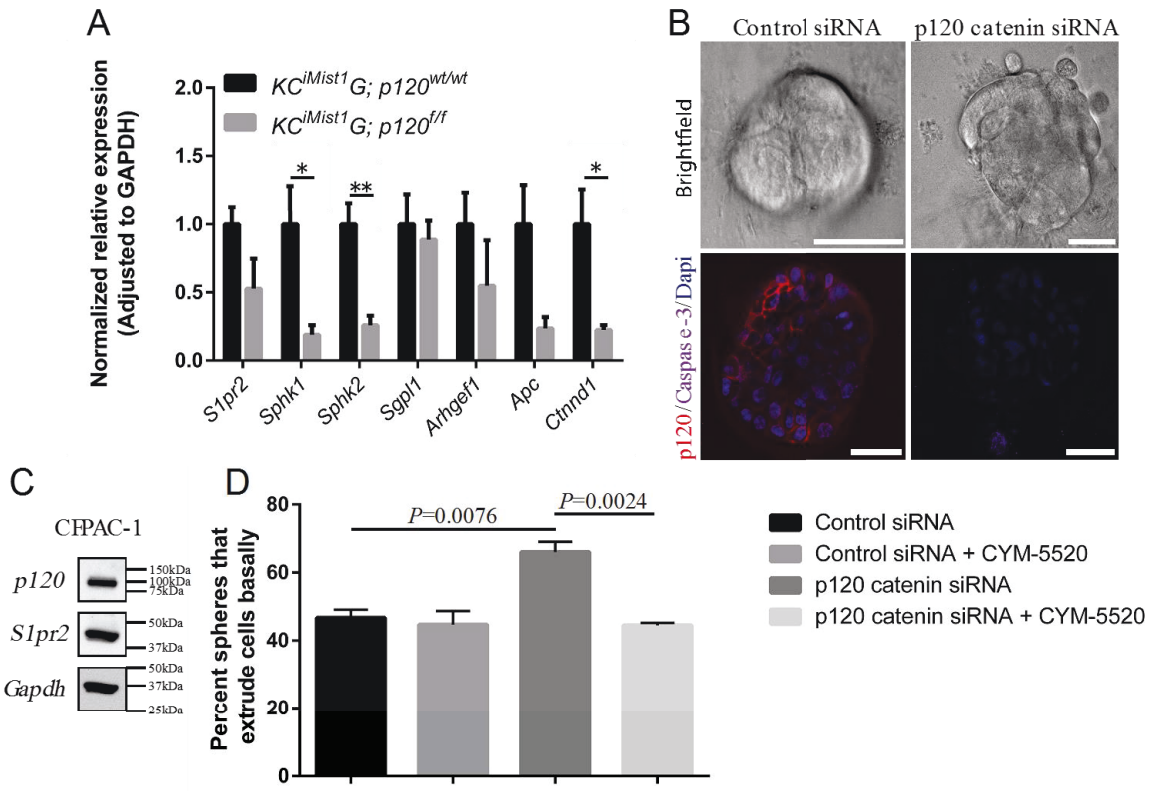

Figure 3.23. A specific S1pr2 agonist completely restores increased epithelial cell extrusion observed with p120 catenin loss. A) qPCR showed significant downregulation of genes involved in the biosynthetic pathway of S1P including *Sphk1* and *Sphk2* in KC^{iMist1}; $p120^{f/f}$ pancreata (n=3 pancreata) when compared to KC^{iMist1}; $p120^{wt/wt}$ pancreata (n=4 pancreata). *Ctnnd1* was significantly decreased in KC^{iMist1}; $p120^{f/f}$ pancreata when compared to KC^{iMist1}; $p120^{wt/wt}$ pancreata. KC^{iMist1}; $p120^{wt/wt}$ relative expression values were normalized to 1 for each gene. B) CFPAC-1 spheres transfected with control and p120 catenin siRNA are shown. IF images show effective p120 catenin knockdown in siRNA treated cultures. Cleaved Caspase-3 labeling shows basal extrusion of a live and dead cell in the siRNA treated CFPAC-1 sphere. C) Western blot depicts expression of p120 catenin and S1pr2 in CFPAC-1 cells. D) A significant increase in CFPAC-1 spheres extruding cells basally was observed in cultures treated with p120 catenin siRNA (66% ± 3.06%) when compared to cultures treated with control siRNA (46.67% ± 2.40%). Addition of the S1pr2 specific agonist CYM-5520 significantly decreased CFPAC-1 spheres extruding cells basally in p120 catenin deficient CFPAC-1 spheres (44.5% ± 0.75%). n=3 experiments for each condition. Scale bars are 50µm.

DISCUSSION

The critical role of the cell-cell adhesion apparatus during tumorigenesis of epithelial cancers is firmly established (Cavallaro & Christofori, 2004; Jones et al., 2008). The association of p120 catenin with E-cadherin at epithelial cell membranes is crucial for formation and maintenance of adherens junctions (Ishiyama et al., 2010). p120 catenin loss or mislocalization can destabilize E-cadherin and affect the adhesive repertoire of the cell and its signal transduction status. In $KC^{iMist1}; p120^{f/f}$ mice, simultaneous p120 catenin and E-cadherin loss likely destabilizes cell adhesion and promotes migration and invasion.

Studies have shown that p120 catenin can function as a *bona fide* tumor suppressor in murine oral cavity, esophagus, and forestomach (Stairs et al., 2011). In contrast, targeted knockout of p120 catenin in murine salivary gland (Davis & Reynolds, 2006), intestine (Perez-Moreno et al., 2006; Smalley-Freed et al., 2011), brain (Elia, Yamamoto, Zang, & Reichardt, 2006), mammary gland (Kurley et al., 2012), kidney (Marciano et al., 2011), eye (Tian, Sanders, Reynolds, van Roy, & van Hengel, 2012), epidermis (Perez-Moreno et al., 2006; Perez-Moreno et al., 2008), and lung (Chignalia et al., 2015) is not sufficient to promote formation of cancer. It is therefore likely that the requirement for p120 catenin in development and progression of cancer is tissue-specific. For pancreas, ablation of p120 catenin in pancreatic progenitor cells in $C^{Pdx1}; p120^{f/f}$ mice (Hendley et al., 2015) and somatic knockout of p120 catenin in adult pancreatic acinar cells in $C^{iMist1}; p120^{f/f}$ mice does not result in development of pancreatic cancer, so evidence for a *bona fide* tumor suppressor role for p120 catenin in pancreas is lacking.

The inflammation suppression function of p120 catenin is well documented (Hu, 2012). Pancreatic loss of p120 catenin generates a microenvironment disposed to chronic inflammation, which may explain in part, the significant increase in ADM and PanIN formation in KC^{iMist1}; $p120^{f/f}$ pancreata when compared to KC^{iMist1}; $p120^{wt/wt}$ pancreata. Cerulein-induced pancreatitis in mice promotes significant increases in ADM and PanIN formation (Guerra et al., 2007; McAllister et al., 2014). Furthermore, dexamethasone, which has anti-inflammatory and immunosuppressant effects, has been shown to reduce ADM and PanIN formation in mice (Rhim et al., 2012), suggesting a potential link between inflammation and premalignant lesion formation.

We show loss of p120 catenin in delaminated cells associated with EMT and non-EMT in PanIN mice. While loss of p120 catenin precedes non-EMT associated delamination in KC^{iMist1}G; $p120^{f/f}$ pancreata, it is unclear if loss of p120 catenin precedes or occurs after delamination associated with EMT in $KPC^{Ptf1a}Y$ and KC^{iMist1}G; $p120^{wt/wt}$ pancreata. Actin cytoskeleton remodeling events are necessary for EMT, and loss of cytoplasmic p120 catenin before occurrence of delamination may prevent cytoskeletal restructuring required for delamination associated with EMT. Biallelic loss of p120 catenin in PanIN mice results in significant downregulation of genes mediating S1P biosynthesis, and activation of S1pr2 *in vitro* completely rescues increased basal epithelial cell extrusion seen with p120 catenin loss. These data suggest that p120 catenin loss in the context of oncogenic Kras may promote neoplastic epithelial cell invasion in part by altering S1P/S1pr2 signaling. Together, altered cytoskeletal organization, decreased cell adhesion, and faulty S1P/S1pr2 signaling may contribute to increased basally extruded epithelial cells (Figure 3.24).

Few mechanisms for non-EMT associated delamination are described, yet the evidence that EMT is not absolutely required for invasion, dissemination, and metastasis is emerging (X. Liu, Huang, Remmers, & Hollingsworth, 2014; Shamir et al., 2014; Tsuji, Ibaragi, & Hu, 2009). In summary, we have created a model in which cooperating genetic insults have unraveled a new mechanism for neoplastic epithelial cell invasion in premalignant pancreatic cancer. The evidence that p120 catenin regulates epithelial cell extrusion is compelling, as p120 catenin loss affects this biologic process in injured, regenerating, and neoplastic pancreata. Further studies are needed to clarify the metastatic potential associated with monoallelic and biallelic p120 catenin loss in different pancreatic cell types and how these characteristics determine the evolution of PDAC.

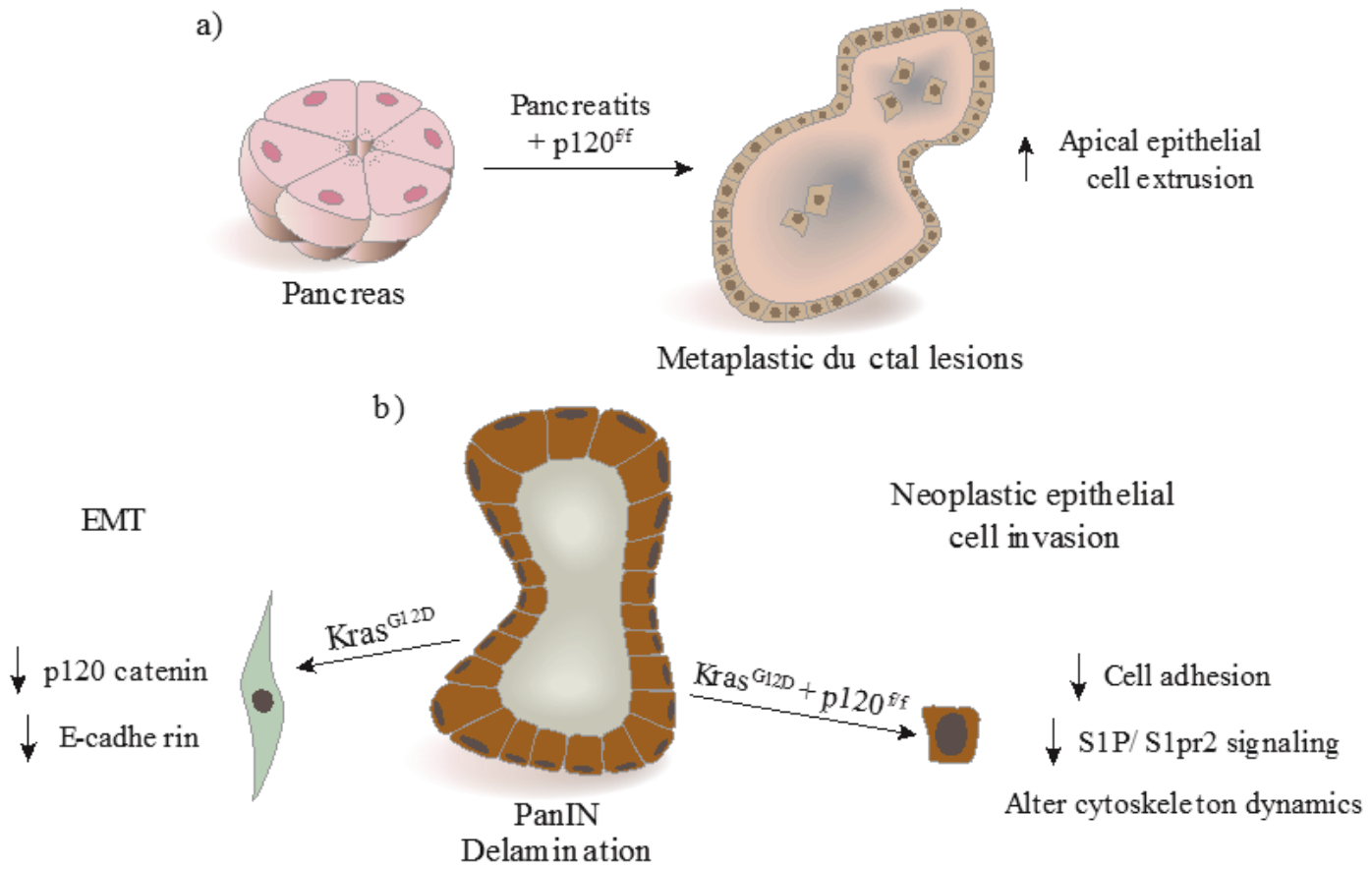

Figure 3.24 - Illustration depicting p120 catenin regulation of epithelial cell extrusion. a) p120 catenin loss in adult pancreatic acinar cells in the context of experimental pancreatitis promotes apical and basal epithelial cell extrusion. b) In the context of oncogenic Kras, PanIN epithelial cells extruding basally through EMT mediated delamination express less adherens junction proteins p120 catenin and E-cadherin. Mutant Kras PanIN epithelial cells deficient for p120 catenin retain epithelial morphology after extruding basally and display features of malignancy. This neoplastic epithelial cell invasion is associated with altered cytoskeletal organization, decreased adherens junctions, and defective S1P/$S1P_2$ signaling.

MATERIALS AND METHODS

Human pancreatic tissue microarrays

For expression analysis, a labeling score of 0-2 corresponding to absent/low, medium, and high was assigned using immunohistochemistry (IHC), and immunofluorescence (IF) staining or IHC was scored for predominant subcellular localization analysis all using human tissue microarrays (TMAs). p120 catenin expression level and predominant subcellular localization were each scored by 3 independent observers blinded to lesion classification. Importantly, each TMA contained normal human pancreatic tissue as a control. IF images were maximum intensity projections of Z-stacks taken using high resolution confocal microscopy allowing visualization of membranous, cytoplasmic, and nuclear labeling. In this study, predominant is defined as greater than or equal to 60% of the representative staining pattern or expression level. Human pancreatic pathologies were classified by a pathologist using H&E stained sections, either serial or exact same section that was scored.

Mice

Transgenic mouse strains *Mist1*$^{CreER/+}$ (*C*^{iMist1}) (Habbe et al., 2008), *Ctnnd1*^{tm1Abre} *(p120*^{f}*)* (Davis & Reynolds, 2006), and *lox-stop-lox; Kras*^{G12D} (*K*) (Hingorani et al., 2003) have been previously described. To perform lineage tracing, we introduced the *Rosa26*^{mTmG} (*G*) (Muzumdar et al., 2007) double fluorescent reporter allele into *KC*^{iMist1}*; p120*$^{wt/wt}$, *KC*^{iMist1}*; p120*$^{f/wt}$, and *KC*^{iMist1}*; p120*$^{f/f}$ lines. Transgenic strains Ptf1atm1(cre)Wri (*C*^{Ptf1a}) (Kawaguchi et al., 2002), lox-stop-lox; KrasG12D (*K*) (Hingorani et al., 2003), p53LoxP (*P*) (Donehower et al., 1992), and R26R-YFP (*Y*) (Srinivas et al., 2001) were used to

generate $KPC^{Ptf1a}Y$ mice. $KPC^{Ptf1a}Y$ mice were maintained on a mixed genetic background, and C^{iMist1}, $p120^{f}$, *K*, and *G* mice were maintained on a C57BL/6J background. For experiments involving KC^{iMist1}mice, all mice were injected at 7-9 weeks of age subcutaneously with 5mg tamoxifen (Sigma, T5648) once per day for 3 consecutive days to induce Cre recombination. For studies involving mice harboring lineaged traced PanIN lesions, $KPC^{Ptf1a}Y$ mice were sacrificed between 6 and 8 weeks of age. Experimental pancreatitis was elicited as previously described (Siveke et al., 2008). Mice were genotyped by PCR or Transnetyx. All pancreatic pathologies in transgenic mice were evaluated by a pathologist. All animal studies were approved by the Animal Care and Use Committee at Johns Hopkins University and University of Texas Health Science Center at Houston.

Histology/immunostaining

Tissues were fixed in either 10% neutral buffered formalin or 4% paraformaldehyde at 4°C, processed according to standard protocols, and embedded in paraffin for sectioning. 5 micron sections were prepared for H&E staining, IHC, and IF. Antigen retrieval was performed for all antibodies using heat-mediated microwave antigen retrieval and a citrate-based, antigen unmasking solution from Vector Laboratories (H-3300) except rat anti-CD45 (Abcam, ab185744). For rat-anti-CD45, Retrievit 6 antigen retrieval solution was used (BioGenex, BS-1006-00). All sections were blocked in 10% FBS in PBST and primary antibodies were incubated overnight at 4°C. Secondary antibodies, from Jackson Immunoresearch, were used at 1:250 and incubated at room temperature for 2 hours. For IF, slides were stained with IHC-Tek Dapi counterstain solution (IHC World, IW-1404)

and mounted in fluorescence mounting medium (Dako, S3023). Immunofluorescent tissues were visualized on a Nikon A1R confocal microscope system. For IHC, slides were developed using Vectastain Elite ABC kit (Vector Laboratories, PK-6100) and DAB Peroxidase (HRP) Substrate kit (Vector Laboratories, SK-4100) and counterstained with hematoxylin. Primary antibodies used in this study: rabbit anti-α-Amylase (Abcam, ab125230, 1:500), mouse anti-β-catenin (BD Transduction Laboratories, 610154, 1:800), rabbit anti-cleaved Caspase-3 (Cell Signaling Technology, 9664S, 1:200), mouse anti-Cytokeratin 19 (MyBioSource, MBS850502, 1:200), rat anti-CD45 (Abcam, ab185744, 1:250), rat anti-Cytokeratin 19 (Developmental Studies Hybridoma Bank, Troma-III, 1:100), rabbit anti-Cytokeratin 19 (Epitomics, AC-0073, 1:300), mouse anti-E-cadherin (BD Transduction Laboratories, 610181, 1:500), chicken anti-GFP (Abcam, ab13970, 1:250), rabbit anti-Laminin (Abcam, ab11575, 1:200), rabbit anti-NF-κB p65 (phospho S536) (Abcam, ab28856, 1:50), rabbit anti-p120 Catenin (Genetex, GTX62350, 1:200), rabbit anti-p120 Catenin (Spring Bioscience, M3630, 1:100), rabbit anti-PKCζ (Santa Cruz, sc-216, 1:500), mouse anti-pp120 (BD Biosciences, 610133, 1:400), and chicken anti-Vimentin (Millipore, AB5733, 1:400).

Premalignant lesion quantification

ADM, PanIN1, PanIN2/3, and fibrostroma were quantified using morphometric analysis on hematoxylin and eosin (H&E) scanned slides in ImageJ. 2 sections per animal sampled at least 400μm apart were analyzed. Quantification of pancreatic area excluded lymph nodes. Slides were scanned using an Aperio ScanScope AT.

CK19 quantification

For quantification of $CK19^+$ basal cell extrusion, $CK19^+$ cells (excluding apically extruded $CK19^+$ cells and normal pancreatic ducts) were counted in 1 scanned section per animal. For quantification of $CK19^+$ apical cell extrusion, $CK19^+$ cells that comprised a luminal pancreatic epithelial structure (lumen sized at least twice the diameter of a cell comprising the epithelial structure and excluding normal ducts) and its associated apically extruded $CK19^+$ cells were counted. 1 scanned section per animal was analyzed for apical extrusion quantification. Slides were scanned using an Aperio ScanScope AT.

Quantification of cerulean-induced pancreatic injury

Pancreatic injury, defined as area containing metaplastic duct lesions and/or inflammation, was quantified in 1 scanned H&E stained section per animal using morphometric analysis in ImageJ. Quantification of pancreatic area excluded lymph nodes. Slides were scanned using an Aperio ScanScope AT.

DNA ploidy analysis

DNA ploidy cell cycle analysis of basally extruded single epithelial cells on 4µm thick Feulgen-stained sections was accomplished using OTMIAS Version 2.0 Image Analysis Software by Olive Tree Media, LLC. Serial sections stained by CK19 IHC were used for identification of isolated, basally extruded epithelial cells in Feulgen stained sections. The internal reference control and isolated epithelial cells analyzed were located on the same Feulgen stained section. An aneuploid peak is defined as any distinct peak with a

DNA index > 1.25. Abnormal DNA content is defined as any aneuploid peak or any peak ≥5C.

RNA isolation, microarray, and qPCR

Adult pancreatic cells were dissociated as previously described (Alsina, Leach, & Bailey, 2013). RNA was isolated from sorted GFP^+ cells using Arcterus PicoPure RNA isolation kit and gene expression was analyzed using Mouse exon microarray 1.0 ST (Affymetrix). For qPCR experiments, reverse transcription was accomplished using QuantiTect reverse transcription kit (Qiagen, 205311). Complementary DNA was amplified using TaqMan gene expression assays (Life Technologies) and the Roche LightCycler 480 system. Expression levels were normalized against mouse GAPDH.

Western blotting

CFPAC-1 cell extracts were prepared according to standard protocols using cell lysis buffer (Cell Signaling Technology, 9803S) with protease inhibitor cocktail tablets (Roche, 4693159001) and 100 mM PMSF. Membranes were incubated overnight with anti-pp120 (BD Biosciences, 610133, 1:500) and anti-EDG-5 (antibodies-online, ABIN317633, 1:1000). GAPDH (Cell Signaling Technology, 5174S, 1:5000) was used as an internal control. After incubation with the respective HRP-conjugated secondary antibody at RT for 1 hour, membranes were developed using either the SuperSignal West Pico Chemiluminescent Substrate (Thermo scientific, 34080) or the SuperSignal West Femto Maximum Sensitivity Substrate (Thermo scientific, 34095).

Cell culture/immunostaining

CFPAC-1 cells were cultured in Dulbecco's minimum essential medium (DMEM), low glucose, GlutaMAX™ Supplement (Life Technologies, 10567-022) supplemented with 10% FBS (Sigma-Aldrich, F4135-500ML) and 1X penicillin-streptomycin-glutamine (Fisher Scientific, 10378-016) at 5% CO_2, 37°C. For culturing CFPAC-1 cells in Matrigel, a single cell suspension of CFPAC-1 cells was resuspended in 4% Matrigel (BD Biosciences, 356234) at a final concentration of 6 x 10^4 cells/mL. 300µL cells per well was placed in 24 well glass bottom plates (In Vitro Scientific, P24-1.5H-N) coated with a thin polymerized layer of Matrigel. For immunostaining, cells were fixed in 4% paraformaldehyde in PBS at 37°C for 20 minutes, permeabilized for 10 minutes with 0.2% Triton X-100, blocked with 10% FBS in PBST for 30 minutes, and incubated with primary antibodies mouse anti-pp120 (BD Biosciences, 610133, 1:400) and rabbit anti-cleaved Caspase-3 (Cell Signaling Technology, 9664S, 1:100) diluted in blocking buffer for 2 hours. Subsequently, cells were incubated with secondary antibodies (Jackson Immunoresearch) diluted in blocking buffer at RT for 2 hours. Dapi was used for nuclear staining and cells were mounted in fluorescence mounting medium (Dako, S3023). Immunofluorescent CFPAC-1 spheres were visualized on a Nikon A1R confocal microscope system.

Transfection/drug treatment

CFPAC-1 cells grown in Matrigel formed spheres by Day 2 and were transfected on Day 2 using lipofectamine RNAiMAX (Invitrogen, 56532). The final concentration of siRNA used per well was 5pmol. siRNA against p120 catenin and control siRNA were obtained

from Santa Cruz (sc-36139 and sc-37007, respectively). For S1pr2 agonist experiments, cells were treated with 10μM CYM-5520 (Sigma, SML1014-25MG) on Day 4, 48 hours after transfection. CFPAC-1 spheres were fixed on Day 6, 96 hours after transfection, for analysis. Day 0 is defined as the day of plating the cells in Matrigel.

Accession number

The Gene Expression Omnibus accession number for the microarray analysis reported in this paper is GSE68090.

Statistical analysis

Data are presented as mean ± SEM and were analyzed in GraphPad Prism or Microsoft Office Excel. Statistical significance was assumed at a *P* value of ≤ 0.05. *P* values were calculated with the unpaired *t*-test unless indicated otherwise. For interpretation of statistical results from unpaired *t*-test, * = p value ≤ 0.05, ** = p value ≤ 0.01, *** = p value ≤ 0.001, and **** = p value ≤ 0.0001.

CHAPTER 4 - CONCLUSION

The results described herein establish important biological functions of p120 catenin in pancreatic morphogenesis, homeostasis, and tumorigenesis. The mouse models generated in this study display both deficiency in p120 catenin and a reduction of adherens junction molecules allowing a unique model system to examine the biology of adherens junctions *in vivo*. We have shown that biallelic loss of p120 catenin during pancreatic development impaired pancreatic tubulogenesis and branching morphogenesis and manifested a biological role for p120 catenin in maintenance of adult pancreatic main ductal epithelium and regulation of pancreatitis. Induction of experimental pancreatitis in p120 catenin depleted pancreata revealed a delayed regeneration capacity, suggesting a functional role for adherens junctions in recovery from pancreatic injury.

Given the previous findings establishing p120 catenin as a tumor suppressor gene in murine oral cavity, squamous forestomach, and esophagus, it was unclear whether p120 catenin would have similar tumor suppressor activities in the pancreas. Development of frank PDAC was not observed with biallelic p120 catenin loss in either mouse model reported herein, suggesting that p120 catenin is not a *bona fide* tumor suppressor in pancreas. The inducible $KC^{iMist1}G;\ p120^{f/f}$ mouse model developed in this study represents a new *in vivo* model to study epithelial cell delamination in early pancreatic neoplasia. The discovery that p120 catenin promotes invasion by regulating S1P/S1pr2 signaling yields important implications for identification of S1P/S1pr2 signaling as a therapeutic target for PDAC. Because prior research has established low level expression of S1pr2 in human PDAC, further studies focused on examination of

small molecules to restore defective S1P/S1pr2 signaling in human PDAC will exponentially amplify potential applications of this therapeutic target.

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REFERENCES

Alsina, J., Leach, S., & Bailey, J. (2013). Incorporation of polaxamers during tissue dissociation allows isolation of high quality RNA from FACS-sorted pancreatic cells. *Pancreapedia: The Exocrine Pancreas Knowledge Base*.

Anastasiadis, P. Z., Moon, S. Y., Thoreson, M. A., Mariner, D. J., Crawford, H. C., Zheng, Y., & Reynolds, A. B. (2000). Inhibition of RhoA by p120 catenin. *Nat Cell Biol, 2*(9), 637-644. doi:10.1038/35023588

Andrade, D., & Rosenblatt, J. (2011). Apoptotic regulation of epithelial cellular extrusion. *Apoptosis, 16*(5), 491-501. doi:10.1007/s10495-011-0587-z

Bailey, J. M., Alsina, J., Rasheed, Z. A., McAllister, F. M., Fu, Y. Y., Plentz, R., . . . Leach, S. D. (2014). DCLK1 marks a morphologically distinct subpopulation of cells with stem cell properties in preinvasive pancreatic cancer. *Gastroenterology, 146*(1), 245-256. doi:10.1053/j.gastro.2013.09.050

Baluch, D. P., & Capco, D. G. (2008). GSK3 beta mediates acentromeric spindle stabilization by activated PKC zeta. *Dev Biol, 317*(1), 46-58. doi:10.1016/j.ydbio.2008.01.044

Cavallaro, U., & Christofori, G. (2004). Cell adhesion and signalling by cadherins and Ig-CAMs in cancer. *Nat Rev Cancer, 4*(2), 118-132. doi:10.1038/nrc1276

Chaw, S. Y., Majeed, A. A., Dalley, A. J., Chan, A., Stein, S., & Farah, C. S. (2012). Epithelial to mesenchymal transition (EMT) biomarkers--E-cadherin, beta-catenin, APC and Vimentin--in oral squamous cell carcinogenesis and transformation. *Oral Oncol, 48*(10), 997-1006. doi:10.1016/j.oraloncology.2012.05.011

Chetty, R., Jain, D., & Serra, S. (2008). p120 catenin reduction and cytoplasmic relocalization leads to dysregulation of E-cadherin in solid pseudopapillary tumors of the pancreas. *Am J Clin Pathol, 130*(1), 71-76. doi:10.1309/FEYD99TXC4LMYVA5

Chignalia, A. Z., Vogel, S. M., Reynolds, A. B., Mehta, D., Dull, R. O., Minshall, R. D., . . . Liu, Y. (2015). p120-Catenin Expressed in Alveolar Type II Cells Is Essential for the Regulation of Lung Innate Immune Response. *Am J Pathol*. doi:10.1016/j.ajpath.2015.01.022

Clevers, H. (2006). Wnt/beta-catenin signaling in development and disease. *Cell, 127*(3), 469-480. doi:10.1016/j.cell.2006.10.018

Davis, M. A., Ireton, R. C., & Reynolds, A. B. (2003). A core function for p120-catenin in cadherin turnover. *J Cell Biol, 163*(3), 525-534. doi:10.1083/jcb.200307111

Davis, M. A., & Reynolds, A. B. (2006). Blocked acinar development, E-cadherin reduction, and intraepithelial neoplasia upon ablation of p120-catenin in the mouse salivary gland. *Dev Cell, 10*(1), 21-31. doi:10.1016/j.devcel.2005.12.004

Dessimoz, J., Bonnard, C., Huelsken, J., & Grapin-Botton, A. (2005). Pancreas-specific deletion of beta-catenin reveals Wnt-dependent and Wnt-independent functions during development. *Curr Biol, 15*(18), 1677-1683. doi:10.1016/j.cub.2005.08.037

Donehower, L. A., Harvey, M., Slagle, B. L., McArthur, M. J., Montgomery, C. A., Jr., Butel, J. S., & Bradley, A. (1992). Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. *Nature, 356*(6366), 215-221. doi:10.1038/356215a0

Ebnet, K., Suzuki, A., Horikoshi, Y., Hirose, T., Meyer Zu Brickwedde, M. K., Ohno, S., & Vestweber, D. (2001). The cell polarity protein ASIP/PAR-3 directly associates with junctional adhesion molecule (JAM). *EMBO J, 20*(14), 3738-3748. doi:10.1093/emboj/20.14.3738

Eisenhoffer, G. T., Loftus, P. D., Yoshigi, M., Otsuna, H., Chien, C. B., Morcos, P. A., & Rosenblatt, J. (2012). Crowding induces live cell extrusion to maintain homeostatic cell numbers in epithelia. *Nature, 484*(7395), 546-549. doi:10.1038/nature10999

Eisenhoffer, G. T., & Rosenblatt, J. (2013). Bringing balance by force: live cell extrusion controls epithelial cell numbers. *Trends Cell Biol, 23*(4), 185-192. doi:10.1016/j.tcb.2012.11.006

Elia, L. P., Yamamoto, M., Zang, K., & Reichardt, L. F. (2006). p120 catenin regulates dendritic spine and synapse development through Rho-family GTPases and cadherins. *Neuron, 51*(1), 43-56. doi:10.1016/j.neuron.2006.05.018

Esni, F., Johansson, B. R., Radice, G. L., & Semb, H. (2001). Dorsal pancreas agenesis in N-cadherin- deficient mice. *Dev Biol, 238*(1), 202-212. doi:10.1006/dbio.2001.0405

Etienne-Manneville, S., & Hall, A. (2001). Integrin-mediated activation of Cdc42 controls cell polarity in migrating astrocytes through PKCzeta. *Cell, 106*(4), 489-498. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11525734

Even-Faitelson, L., & Ravid, S. (2006). PAK1 and aPKCzeta regulate myosin II-B phosphorylation: a novel signaling pathway regulating filament assembly. *Mol Biol Cell, 17*(7), 2869-2881. doi:10.1091/mbc.E05-11-1001

Fei, Y., Cheng, Z., Liu, S., Liu, X., Ge, Z., Wang, F., . . . Wang, W. (2009). Expression and clinical significance of p120 catenin mRNA and protein in pancreatic carcinoma. *Bosn J Basic Med Sci, 9*(3), 191-197. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/19754472

Fleming, T. P., Papenbrock, T., Fesenko, I., Hausen, P., & Sheth, B. (2000). Assembly of tight junctions during early vertebrate development. *Semin Cell Dev Biol, 11*(4), 291-299. doi:10.1006/scdb.2000.0179

Golosow, N., & Grobstein, C. (1962). Epitheliomesenchymal interaction in pancreatic morphogenesis. *Dev Biol, 4*, 242-255. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/13899987

Gomez, J., Martinez de Aragon, A., Bonay, P., Pitton, C., Garcia, A., Silva, A., . . . Rebollo, A. (1995). Physical association and functional relationship between protein kinase C zeta and the actin cytoskeleton. *Eur J Immunol, 25*(9), 2673-2678. doi:10.1002/eji.1830250941

Gopinathan, A., Morton, J. P., Jodrell, D. I., & Sansom, O. J. (2015). GEMMs as preclinical models for testing pancreatic cancer therapies. *Dis Model Mech, 8*(10), 1185-1200. doi:10.1242/dmm.021055

Gu, G., Dubauskaite, J., & Melton, D. A. (2002). Direct evidence for the pancreatic lineage: NGN3+ cells are islet progenitors and are distinct from duct progenitors. *Development, 129*(10), 2447-2457.

Gu, Y., Forostyan, T., Sabbadini, R., & Rosenblatt, J. (2011). Epithelial cell extrusion requires the sphingosine-1-phosphate receptor 2 pathway. *J Cell Biol, 193*(4), 667-676. doi:10.1083/jcb.201010075

Gu, Y., & Rosenblatt, J. (2012). New emerging roles for epithelial cell extrusion. *Curr Opin Cell Biol, 24*(6), 865-870. doi:10.1016/j.ceb.2012.09.003

Gu, Y., Shea, J., Slattum, G., Firpo, M. A., Alexander, M., Mulvihill, S. J., . . . Rosenblatt, J. (2015). Defective apical extrusion signaling contributes to aggressive tumor hallmarks. *Elife, 4*, e04069. doi:10.7554/eLife.04069

Guerra, C., Schuhmacher, A. J., Canamero, M., Grippo, P. J., Verdaguer, L., Perez-Gallego, L., . . . Barbacid, M. (2007). Chronic pancreatitis is essential for induction of pancreatic ductal adenocarcinoma by K-Ras oncogenes in adult mice. *Cancer Cell, 11*(3), 291-302. doi:10.1016/j.ccr.2007.01.012

Guo, H., Gu, F., Li, W., Zhang, B., Niu, R., Fu, L., . . . Ma, Y. (2009). Reduction of protein kinase C zeta inhibits migration and invasion of human glioblastoma cells. *J Neurochem, 109*(1), 203-213. doi:10.1111/j.1471-4159.2009.05946.x

Habbe, N., Shi, G., Meguid, R. A., Fendrich, V., Esni, F., Chen, H., . . . Maitra, A. (2008). Spontaneous induction of murine pancreatic intraepithelial neoplasia (mPanIN) by acinar cell targeting of oncogenic Kras in adult mice. *Proc Natl Acad Sci U S A, 105*(48), 18913-18918. doi:10.1073/pnas.0810097105

Hamada, S., Satoh, K., Miura, S., Hirota, M., Kanno, A., Masamune, A., . . . Shimosegawa, T. (2013). miR-197 induces epithelial-mesenchymal transition in pancreatic cancer cells by targeting p120 catenin. *J Cell Physiol, 228*(6), 1255-1263. doi:10.1002/jcp.24280

Hartsock, A., & Nelson, W. J. (2008). Adherens and tight junctions: structure, function and connections to the actin cytoskeleton. *Biochim Biophys Acta, 1778*(3), 660-669. doi:10.1016/j.bbamem.2007.07.012

Heiser, P. W., Cano, D. A., Landsman, L., Kim, G. E., Kench, J. G., Klimstra, D. S., . . . Hebrok, M. (2008). Stabilization of beta-catenin induces pancreas tumor formation. *Gastroenterology, 135*(4), 1288-1300. doi:10.1053/j.gastro.2008.06.089

Heiser, P. W., Lau, J., Taketo, M. M., Herrera, P. L., & Hebrok, M. (2006). Stabilization of beta-catenin impacts pancreas growth. *Development, 133*(10), 2023-2032. doi:10.1242/dev.02366

Hendley, A. M., Provost, E., Bailey, J. M., Wang, Y. J., Cleveland, M. H., Blake, D., . . . Leach, S. D. (2015). p120 Catenin is required for normal tubulogenesis but not epithelial integrity in developing mouse pancreas. *Dev Biol, 399*(1), 41-53. doi:10.1016/j.ydbio.2014.12.010

Hingorani, S. R., Petricoin, E. F., Maitra, A., Rajapakse, V., King, C., Jacobetz, M. A., . . . Tuveson, D. A. (2003). Preinvasive and invasive ductal pancreatic cancer and its early detection in the mouse. *Cancer Cell, 4*(6), 437-450. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/14706336

Hofmann, I., Schlechter, T., Kuhn, C., Hergt, M., & Franke, W. W. (2009). Protein p0071 - an armadillo plaque protein that characterizes a specific subtype of adherens junctions. *J Cell Sci, 122*(Pt 1), 21-24. doi:10.1242/jcs.043927

Hollingsworth, M. A., & Swanson, B. J. (2004). Mucins in cancer: protection and control of the cell surface. *Nat Rev Cancer, 4*(1), 45-60. doi:10.1038/nrc1251

Hu, G. (2012). p120-Catenin: a novel regulator of innate immunity and inflammation. *Crit Rev Immunol, 32*(2), 127-138. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/23216611

Ireton, R. C., Davis, M. A., van Hengel, J., Mariner, D. J., Barnes, K., Thoreson, M. A., . . . Reynolds, A. B. (2002). A novel role for p120 catenin in E-cadherin function. *J Cell Biol, 159*(3), 465-476. doi:10.1083/jcb.200205115

Ishiyama, N., Lee, S. H., Liu, S., Li, G. Y., Smith, M. J., Reichardt, L. F., & Ikura, M. (2010). Dynamic and static interactions between p120 catenin and E-cadherin regulate the stability of cell-cell adhesion. *Cell, 141*(1), 117-128. doi:10.1016/j.cell.2010.01.017

Izumi, Y., Hirose, T., Tamai, Y., Hirai, S., Nagashima, Y., Fujimoto, T., . . . Ohno, S. (1998). An atypical PKC directly associates and colocalizes at the epithelial tight junction with ASIP, a mammalian homologue of Caenorhabditis elegans polarity protein PAR-3. *J Cell Biol, 143*(1), 95-106. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9763423

Johansson, A., Driessens, M., & Aspenstrom, P. (2000). The mammalian homologue of the Caenorhabditis elegans polarity protein PAR-6 is a binding partner for the Rho GTPases Cdc42 and Rac1. *J Cell Sci, 113 (Pt 18)*, 3267-3275. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10954424

Johansson, J. K., Voss, U., Kesavan, G., Kostetskii, I., Wierup, N., Radice, G. L., & Semb, H. (2010). N-cadherin is dispensable for pancreas development but required for beta-cell granule turnover. *Genesis, 48*(6), 374-381. doi:10.1002/dvg.20628

Jones, S., Zhang, X., Parsons, D. W., Lin, J. C., Leary, R. J., Angenendt, P., . . . Kinzler, K. W. (2008). Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. *Science, 321*(5897), 1801-1806. doi:10.1126/science.1164368

Kawaguchi, Y., Cooper, B., Gannon, M., Ray, M., MacDonald, R. J., & Wright, C. V. (2002). The role of the transcriptional regulator Ptf1a in converting intestinal to pancreatic progenitors. *Nat Genet, 32*(1), 128-134. doi:10.1038/ng959

Kurley, S. J., Bierie, B., Carnahan, R. H., Lobdell, N. A., Davis, M. A., Hofmann, I., . . . Reynolds, A. B. (2012). p120-catenin is essential for terminal end bud function and mammary morphogenesis. *Development, 139*(10), 1754-1764. doi:10.1242/dev.072769

Landsman, L., Nijagal, A., Whitchurch, T. J., Vanderlaan, R. L., Zimmer, W. E., Mackenzie, T. C., & Hebrok, M. (2011). Pancreatic mesenchyme regulates epithelial organogenesis throughout development. *PLoS Biol, 9*(9), e1001143. doi:10.1371/journal.pbio.1001143

Lin, D., Edwards, A. S., Fawcett, J. P., Mbamalu, G., Scott, J. D., & Pawson, T. (2000). A mammalian PAR-3-PAR-6 complex implicated in Cdc42/Rac1 and aPKC

signalling and cell polarity. *Nat Cell Biol, 2*(8), 540-547. doi:10.1038/35019582

Liu, X., Huang, H., Remmers, N., & Hollingsworth, M. A. (2014). Loss of E-cadherin and epithelial to mesenchymal transition is not required for cell motility in tissues or for metastasis. *Tissue Barriers, 2*(4), e969112. doi:10.4161/21688362.2014.969112

Liu, X., Yi, C., Wen, Y., Radhakrishnan, P., Tremayne, J. R., Dao, T., . . . Hollingsworth, M. A. (2014). Interactions between MUC1 and p120 catenin regulate dynamic features of cell adhesion, motility, and metastasis. *Cancer Res, 74*(5), 1609-1620. doi:10.1158/0008-5472.CAN-13-2444

Liu, X. J., Yang, C., Gupta, N., Zuo, J., Chang, Y. S., & Fang, F. D. (2007). Protein kinase C-zeta regulation of GLUT4 translocation through actin remodeling in CHO cells. *J Mol Med (Berl), 85*(8), 851-861. doi:10.1007/s00109-007-0232-z

Mann, K. M., Ward, J. M., Yew, C. C., Kovochich, A., Dawson, D. W., Black, M. A., . . . Copeland, N. G. (2012). Sleeping Beauty mutagenesis reveals cooperating mutations and pathways in pancreatic adenocarcinoma. *Proc Natl Acad Sci U S A, 109*(16), 5934-5941. doi:10.1073/pnas.1202490109

Marciano, D. K., Brakeman, P. R., Lee, C. Z., Spivak, N., Eastburn, D. J., Bryant, D. M., . . . Reichardt, L. F. (2011). p120 catenin is required for normal renal tubulogenesis and glomerulogenesis. *Development, 138*(10), 2099-2109. doi:10.1242/dev.056564

Mariner, D. J., Wang, J., & Reynolds, A. B. (2000). ARVCF localizes to the nucleus and adherens junction and is mutually exclusive with p120(ctn) in E-cadherin complexes. *J Cell Sci, 113 (Pt 8)*, 1481-1490. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10725230

Marshall, T. W., Lloyd, I. E., Delalande, J. M., Nathke, I., & Rosenblatt, J. (2011). The tumor suppressor adenomatous polyposis coli controls the direction in which a cell extrudes from an epithelium. *Mol Biol Cell, 22*(21), 3962-3970. doi:10.1091/mbc.E11-05-0469

Mayerle, J., Friess, H., Buchler, M. W., Schnekenburger, J., Weiss, F. U., Zimmer, K. P., . . . Lerch, M. M. (2003). Up-regulation, nuclear import, and tumor growth stimulation of the adhesion protein p120 in pancreatic cancer. *Gastroenterology, 124*(4), 949-960. doi:10.1053/gast.2003.50142

McAllister, F., Bailey, J. M., Alsina, J., Nirschl, C. J., Sharma, R., Fan, H., . . . Leach, S. D. (2014). Oncogenic Kras Activates a Hematopoietic-to-Epithelial IL-17 Signaling Axis in Preinvasive Pancreatic Neoplasia. *Cancer Cell, 25*(5), 621-637. doi:10.1016/j.ccr.2014.03.014

Murtaugh, L. C., Law, A. C., Dor, Y., & Melton, D. A. (2005). Beta-catenin is essential for pancreatic acinar but not islet development. *Development, 132*(21), 4663-4674. doi:10.1242/dev.02063

Muzumdar, M. D., Tasic, B., Miyamichi, K., Li, L., & Luo, L. (2007). A global double-fluorescent Cre reporter mouse. *Genesis, 45*(9), 593-605. doi:10.1002/dvg.20335

Noren, N. K., Liu, B. P., Burridge, K., & Kreft, B. (2000). p120 catenin regulates the actin cytoskeleton via Rho family GTPases. *J Cell Biol, 150*(3), 567-580. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10931868

O'Konski, M. S., & Pandol, S. J. (1990). Effects of caerulein on the apical cytoskeleton of the pancreatic acinar cell. *J Clin Invest, 86*(5), 1649-1657. doi:10.1172/JCI114887

Pan, S., Chen, R., Reimel, B. A., Crispin, D. A., Mirzaei, H., Cooke, K., . . . Brentnall, T. A. (2009). Quantitative proteomics investigation of pancreatic intraepithelial neoplasia. *Electrophoresis, 30*(7), 1132-1144. doi:10.1002/elps.200800752

Perez-Moreno, M., Davis, M. A., Wong, E., Pasolli, H. A., Reynolds, A. B., & Fuchs, E. (2006). p120-catenin mediates inflammatory responses in the skin. *Cell, 124*(3), 631-644. doi:10.1016/j.cell.2005.11.043

Perez-Moreno, M., & Fuchs, E. (2006). Catenins: keeping cells from getting their signals crossed. *Dev Cell, 11*(5), 601-612. doi:10.1016/j.devcel.2006.10.010

Perez-Moreno, M., Song, W., Pasolli, H. A., Williams, S. E., & Fuchs, E. (2008). Loss of p120 catenin and links to mitotic alterations, inflammation, and skin cancer. *Proc Natl Acad Sci U S A, 105*(40), 15399-15404. doi:10.1073/pnas.0807301105

Pictet, R. L., Clark, W. R., Williams, R. H., & Rutter, W. J. (1972). An ultrastructural analysis of the developing embryonic pancreas. *Dev Biol, 29*(4), 436-467. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/4570759

Pieters, T., van Hengel, J., & van Roy, F. (2012). Functions of p120ctn in development and disease. *Front Biosci (Landmark Ed), 17*, 760-783. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/22201773

Puri, S., & Hebrok, M. (2007). Dynamics of embryonic pancreas development using real-time imaging. *Dev Biol, 306*(1), 82-93. doi:10.1016/j.ydbio.2007.03.003

Qiu, R. G., Abo, A., & Steven Martin, G. (2000). A human homolog of the C. elegans polarity determinant Par-6 links Rac and Cdc42 to PKCzeta signaling and cell transformation. *Curr Biol, 10*(12), 697-707. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10873802

Rakonczay, Z., Jr., Hegyi, P., Takacs, T., McCarroll, J., & Saluja, A. K. (2008). The role of NF-kappaB activation in the pathogenesis of acute pancreatitis. *Gut, 57*(2), 259-267. doi:10.1136/gut.2007.124115

Rhim, A. D., Mirek, E. T., Aiello, N. M., Maitra, A., Bailey, J. M., McAllister, F., . . . Stanger, B. Z. (2012). EMT and dissemination precede pancreatic tumor formation. *Cell, 148*(1-2), 349-361. doi:10.1016/j.cell.2011.11.025

Rosenblatt, J., Raff, M. C., & Cramer, L. P. (2001). An epithelial cell destined for apoptosis signals its neighbors to extrude it by an actin- and myosin-dependent mechanism. *Curr Biol, 11*(23), 1847-1857. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11728307

Rovira, M., Scott, S. G., Liss, A. S., Jensen, J., Thayer, S. P., & Leach, S. D. (2010). Isolation and characterization of centroacinar/terminal ductal progenitor cells in adult mouse pancreas. *Proc Natl Acad Sci U S A, 107*(1), 75-80. doi:10.1073/pnas.0912589107

Rutter, W. J., Kemp, J. D., Bradshaw, W. S., Clark, W. R., Ronzio, R. A., & Sanders, T. G. (1968). Regulation of specific protein synthesis in cytodifferentiation. *J Cell Physiol, 72*(2), Suppl 1:1-18. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/5693298

Satsu, H., Schaeffer, M. T., Guerrero, M., Saldana, A., Eberhart, C., Hodder, P., . . . Brown, S. J. (2013). A sphingosine 1-phosphate receptor 2 selective allosteric agonist. *Bioorg Med Chem, 21*(17), 5373-5382. doi:10.1016/j.bmc.2013.06.012

Schackmann, R. C., Klarenbeek, S., Vlug, E. J., Stelloo, S., van Amersfoort, M., Tenhagen, M., . . . Derksen, P. W. (2013). Loss of p120-catenin induces metastatic progression of breast cancer by inducing anoikis resistance and augmenting growth factor receptor signaling. *Cancer Res, 73*(15), 4937-4949. doi:10.1158/0008-5472.CAN-13-0180

Seymour, P. A., Freude, K. K., Tran, M. N., Mayes, E. E., Jensen, J., Kist, R., . . . Sander, M. (2007). SOX9 is required for maintenance of the pancreatic progenitor cell pool. *Proc Natl Acad Sci U S A, 104*(6), 1865-1870. doi:10.1073/pnas.0609217104

Shamir, E. R., Pappalardo, E., Jorgens, D. M., Coutinho, K., Tsai, W. T., Aziz, K., . . . Ewald, A. J. (2014). Twist1-induced dissemination preserves epithelial identity and requires E-cadherin. *J Cell Biol, 204*(5), 839-856. doi:10.1083/jcb.201306088

Shapiro, L., & Weis, W. I. (2009). Structure and biochemistry of cadherins and catenins. *Cold Spring Harb Perspect Biol, 1*(3), a003053. doi:10.1101/cshperspect.a003053

Siveke, J. T., Lubeseder-Martellato, C., Lee, M., Mazur, P. K., Nakhai, H., Radtke, F., & Schmid, R. M. (2008). Notch signaling is required for exocrine regeneration after acute pancreatitis. *Gastroenterology, 134*(2), 544-555. doi:10.1053/j.gastro.2007.11.003

Slattum, G., Gu, Y., Sabbadini, R., & Rosenblatt, J. (2014). Autophagy in oncogenic K-Ras promotes basal extrusion of epithelial cells by degrading S1P. *Curr Biol, 24*(1), 19-28. doi:10.1016/j.cub.2013.11.029

Slattum, G., McGee, K. M., & Rosenblatt, J. (2009). P115 RhoGEF and microtubules decide the direction apoptotic cells extrude from an epithelium. *J Cell Biol, 186*(5), 693-702. doi:10.1083/jcb.200903079

Slattum, G. M., & Rosenblatt, J. (2014). Tumour cell invasion: an emerging role for basal epithelial cell extrusion. *Nat Rev Cancer, 14*(7), 495-501. doi:10.1038/nrc3767

Smalley-Freed, W. G., Efimov, A., Burnett, P. E., Short, S. P., Davis, M. A., Gumucio, D. L., . . . Reynolds, A. B. (2010). p120-catenin is essential for maintenance of barrier function and intestinal homeostasis in mice. *J Clin Invest, 120*(6), 1824-1835. doi:10.1172/JCI41414

Smalley-Freed, W. G., Efimov, A., Short, S. P., Jia, P., Zhao, Z., Washington, M. K., . . . Reynolds, A. B. (2011). Adenoma formation following limited ablation of p120-catenin in the mouse intestine. *PloS one, 6*(5), e19880. doi:10.1371/journal.pone.0019880

Srinivas, S., Watanabe, T., Lin, C. S., William, C. M., Tanabe, Y., Jessell, T. M., & Costantini, F. (2001). Cre reporter strains produced by targeted insertion of EYFP and ECFP into the ROSA26 locus. *BMC Dev Biol, 1*, 4. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11299042

Stairs, D. B., Bayne, L. J., Rhoades, B., Vega, M. E., Waldron, T. J., Kalabis, J., . . . Rustgi, A. K. (2011). Deletion of p120-catenin results in a tumor microenvironment with inflammation and cancer that establishes it as a tumor suppressor gene. *Cancer Cell, 19*(4), 470-483. doi:10.1016/j.ccr.2011.02.007

Su, Y., Li, J., Shi, C., Hruban, R. H., & Radice, G. L. (2015). N-cadherin functions as a growth suppressor in a model of K-ras-induced PanIN. *Oncogene*. doi:10.1038/onc.2015.382

Su, Y., Li, J., Witkiewicz, A. K., Brennan, D., Neill, T., Talarico, J., & Radice, G. L. (2012). N-cadherin haploinsufficiency increases survival in a mouse model of pancreatic cancer. *Oncogene, 31*(41), 4484-4489. doi:10.1038/onc.2011.574

Tian, H., Sanders, E., Reynolds, A., van Roy, F., & van Hengel, J. (2012). Ocular anterior segment dysgenesis upon ablation of p120 catenin in neural crest cells. *Invest Ophthalmol Vis Sci, 53*(9), 5139-5153. doi:10.1167/iovs.12-9472

Tsuji, T., Ibaragi, S., & Hu, G. F. (2009). Epithelial-mesenchymal transition and cell cooperativity in metastasis. *Cancer Res, 69*(18), 7135-7139. doi:10.1158/0008-5472.CAN-09-1618

Uberall, F., Hellbert, K., Kampfer, S., Maly, K., Villunger, A., Spitaler, M., . . . Grunicke, H. H. (1999). Evidence that atypical protein kinase C-lambda and atypical protein kinase C-zeta participate in Ras-mediated reorganization of the F-actin cytoskeleton. *J Cell Biol, 144*(3), 413-425. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9971737

Valer Carstea, B., Catunda Lemos, A. P., Ilie, E. D., Varga, L., Bodo, S., Kovacs, A., . . . Gocza, E. (2007). Production of identical mouse twins and a triplet with predicted gender. *Cloning Stem Cells, 9*(2), 247-256. doi:10.1089/clo.2006.0055

van Hengel, J., & van Roy, F. (2007). Diverse functions of p120ctn in tumors. *Biochim Biophys Acta, 1773*(1), 78-88. doi:10.1016/j.bbamcr.2006.08.033

Villasenor, A., Chong, D. C., Henkemeyer, M., & Cleaver, O. (2010). Epithelial dynamics of pancreatic branching morphogenesis. *Development, 137*(24), 4295-4305. doi:10.1242/dev.052993

Walter, B., Schlechter, T., Hergt, M., Berger, I., & Hofmann, I. (2008). Differential expression pattern of protein ARVCF in nephron segments of human and mouse kidney. *Histochem Cell Biol, 130*(5), 943-956. doi:10.1007/s00418-008-0456-1

Wells, J. M., Esni, F., Boivin, G. P., Aronow, B. J., Stuart, W., Combs, C., . . . Lowy, A. M. (2007). Wnt/beta-catenin signaling is required for development of the exocrine pancreas. *BMC Dev Biol, 7*, 4. doi:10.1186/1471-213X-7-4

Wu, J., Jiao, Y., Dal Molin, M., Maitra, A., de Wilde, R. F., Wood, L. D., . . . Vogelstein, B. (2011). Whole-exome sequencing of neoplastic cysts of the pancreas reveals recurrent mutations in components of ubiquitin-dependent pathways. *Proc Natl Acad Sci U S A, 108*(52), 21188-21193. doi:10.1073/pnas.1118046108

Wu, J., Mariner, D. J., Thoreson, M. A., & Reynolds, A. B. (1998). Production and characterization of monoclonal antibodies to the catenin p120ctn. *Hybridoma, 17*(2), 175-183. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9627058

Wulf, E., Deboben, A., Bautz, F. A., Faulstich, H., & Wieland, T. (1979). Fluorescent phallotoxin, a tool for the visualization of cellular actin. *Proc Natl Acad Sci U S A, 76*(9), 4498-4502. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/291981

Yap, A. S., Gomez, G. A., & Parton, R. G. (2015). Adherens Junctions Revisualized: Organizing Cadherins as Nanoassemblies. *Dev Cell, 35*(1), 12-20. doi:10.1016/j.devcel.2015.09.012

Zhang, Y., Morris, J. P. t., Yan, W., Schofield, H. K., Gurney, A., Simeone, D. M., . . . Pasca di Magliano, M. (2013). Canonical wnt signaling is required for pancreatic carcinogenesis. *Cancer Res, 73*(15), 4909-4922. doi:10.1158/0008-5472.CAN-12-4384

Zhao, Z. M., Reynolds, A. B., & Gaucher, E. A. (2011). The evolutionary history of the catenin gene family during metazoan evolution. *BMC Evol Biol, 11*, 198. doi:10.1186/1471-2148-11-198

curriculum vitae

Audrey M. Hendley

PRESENT TITLE: Ph.D. student in Human Genetics and Molecular Biology
Johns Hopkins University School of Medicine

PERSONAL: Born**:** Omaha, Nebraska, 1987

CITIZENSHIP: USA

EDUCATION:

Ph.D. Human Genetics and Molecular Biology, Johns Hopkins University School of Medicine Thesis advisor: Steven D. Leach, MD Co-mentor: Jennifer M. Bailey, MA, PhD	Baltimore, MD 2016
B.S. Biology (Major), Chemistry (Minor), University of South Carolina, Aiken, SC *Summa cum laude* Thesis advisor: William H. Jackson, PhD	Aiken, SC 2009

RESEARCH EXPERIENCE:

Graduate Student Johns Hopkins University School of Medicine Advisor: Steven D. Leach, MD Co-mentor: Jennifer M. Bailey, MA, PhD Adherens junctions organize the pancreatic developmental program and manage the evolution of neoplastic epithelium	Baltimore, MD 2009 - 2014 Houston, TX 2014 - 2016
Student Employee University of South Carolina Aiken Advisor: Chad L. Leverette, PhD Investigation of glancing angle vapor deposition (GLAD) for the fabrication of nanostructured SEIRA-active substrates	Aiken, SC 2008
Undergraduate Research University of South Carolina Aiken Advisor: William H. Jackson, PhD Investigating HIV-1 Vif activity using hammerhead ribozymes	Aiken, SC 2006 - 2009

HONORS AND AWARDS:

- *Women in Pancreas travel award*, American Pancreatic Association Annual Meeting, 2015

- *Travel award*, Gordon Research Conference on Pancreatic Diseases, 2015
- *Outstanding Senior Student* (University's highest honor given to 1 selected student per graduating class; accolade responsibilities include student speech at commencement ceremony), University of South Carolina Aiken, 2009
- *Biology Student of the Year* (Department of Biology and Geology's highest honor given to 1 selected student each year), University of South Carolina Aiken, 2009
- *President's Honor List* (all semesters), University of South Carolina Aiken, 2005-2009
- *Legislative Incentive for Future Excellence (LIFE) Scholarship*, South Carolina Commission on Higher Education, 2005-2009
- *University of South Carolina Aiken Academic Scholarship*, 2005-2009
- *Magellan Research Scholarship*, University of South Carolina Columbia, 2008

PUBLICATIONS:

A. *Peer reviewed publications*

Hendley, AM, Wang, YJ, Alsina, J, Ahmed, I, Lafaro, KJ, Zhang, H, Roy, N, Savidge, S, Cao, Y, Hebrok, M, Maitra, A, Reynolds, AB, Goggins, M, Younes, M, Iacobuzio-Donahue, CA, Leach, SD* and Bailey, JM*. 2015. p120 Catenin suppresses basal epithelial cell extrusion in invasive pancreatic neoplasia. ***Cancer Research.*** In revision. (*Co-senior and co-corresponding author)

Choi, E, **Hendley, AM**, Bailey, JM, Leach, SD, Goldenring, JR. 2015. Expression of Activated Ras in Gastric Chief Cells of Mice Leads to the Full Spectrum of Metaplastic Lineage Transitions**.** ***Gastroenterology.*** Advance online publication, 8 Dec. 2015; doi: 10.1053/j.gastro.2015.11.049. PMID: 26677984

Bailey, JM, **Hendley, AM**, Lafaro, KJ, Pruski, MA, Jones, NC, Alsina, J, Younes, M, Maitra, A, McAllister, F, Iacobuzio-Donahue, CA, Leach, SD. p53 gain-of-function mutations promote adenocarcinoma from pancreatic ductal cells. ***Oncogene.*** Advance online publication, 23 Nov. 2015; doi:10.1038/onc.2015.441. PMID: 26592447

Hendley, AM, Provost, E, Bailey, JM, Wang, YJ, Cleveland, MH, Blake, D , Bittman, RW, Roeser, JC, Maitra, A, Reynolds, AB, Leach, SD. 2015. p120 Catenin is required for normal tubulogenesis but not epithelial integrity in developing mouse pancreas. ***Dev. Biol.*** 399(1), 41-53. PMID: 25523391

Wang YJ, McAllister F, Bailey JM, Scott S-G, **Hendley AM**, Leach SD, Ghosh, B. 2014. *Dicer* Is Required for Maintenance of Adult Pancreatic Acinar Cell Identity and Plays a Role in Kras-Driven Pancreatic Neoplasia. ***PLoS ONE.*** 9(11): e113127. doi:10.1371/journal.pone.0113127. PMCID: PMC4236134

B. ***Reviews, chapters, monographs, and editorials***

Bailey, JM*, **Hendley, AM*,** Maitra, A. 2015. New Insights Into Plasticity of Pancreatic Cancer: Cancer to Acinar Cell Reprogramming by the Basic Helix-Loop-Helix Transcription Factor E47. ***Pancreas.*** 44(5), 683-685. (*Co-first author) PMID: 26061556

Hendley, AM and Bailey, JM. 2015. ***Principles of Stem Cell Biology and Cancer: Future Applications and Therapeutics.*** 1 ed. Regad T, Sayers TJ, Rees RC, editors. Hoboken, NJ: Wiley-Blackwell. Chapter 11, Stem Cells and Pancreatic Cancer; p.213-229. 376p.

C. *Published Abstracts*

Hendley, AM, Wang, YJ, Alsina, J, Ahmed, I, Zhang, H, Savidge, S, Ho, H, Reynolds, A, Maitra, A, Goggins, M, Iacobuzio-Donahue, CA, Leach, SD, Bailey, JM. p120 catenin: A novel regulator of epithelial cell delamination in early Kras-driven pancreatic cancer. [abstract]. In: Proceedings of the 106th Annual Meeting of the American Association for Cancer Research; 2015 Apr 18-22; Philadelphia, PA. Philadelphia (PA): AACR; ***Cancer Research*** 2015;75(15 Suppl):Abstract nr 4186. doi:10.1158/1538-7445.AM2015-4186.

Bailey JM, **Hendley AM**, Talbot C, Iacobuzio-Donahue CA, Leach SD. 2015. Even Pancreatic Duct Cells Can Serve As a Cell of Origin for Pancreatic Cancer: Requirement for Cooperating Genetic Lesions. Digestive Disease Week Annual Meeting; Washington, DC. ***Gastroenterology*** 148(4) S-1192-S-1193. DOI: 10.1016/S0016-5085(15)34074-9.

Choi E, **Hendley AM**, Bailey JM, Leach SD, Goldenring JR. 2015. Inhibition of KRAS Activation Ameliorates Pre-Cancerous Metaplastic Conversion in the Mouse Stomach. Digestive Disease Week Annual Meeting; Washington, DC. ***Gastroenterology*** 148(4) Supplement 1, S-5. DOI: http://dx.doi.org/10.1016/S0016-5085(15)30011-1.

Choi, E, **Hendley AM**, Petersen, C, Weis, V, Leach SD, Goldenring, J. 2014. 266 Active Kras leads to rapid transdifferentiation of gastric chief cells into metaplasia with progression to dysplasia and invasive cancer. Digestive Disease Week Annual Meeting, Chicago, IL. ***Gastroenterology*** 146(5) Supplement 1, S-64. DOI: 10.1016/S0016-5085(14)60225-0.

Lafaro, KJ, **Hendley AM**, Bailey, JM, Leach SD. Clonal composition and clonal selection during PanIN progression. [abstract]. In: Proceedings of the AACR Special Conference on Pancreatic Cancer: Innovations in Research and Treatment; May 18-21, 2014; New Orleans, LA. Philadelphia (PA): AACR; *Cancer Research* 2015;75(13 Suppl):Abstract nr PR04. **doi: 10.1158/1538-7445.PANCA2014-PR04**

Bailey, JM, Alsina, J, McAllister, F, **Hendley, AM**, Lafaro, KJ, Habibulla, A, Marchionni, L, Maitra, A, Leach, SD. In search of Kras resistance genes: Whole transcriptome analysis identifies critical pathways mediating resistance and sensitivity to oncogenic Kras. [abstract]. In: Proceedings of the AACR Special Conference on Pancreatic Cancer: Innovations in Research and Treatment; May 18-21, 2014; New

Orleans, LA. Philadelphia (PA): AACR; ***Cancer Research*** 2015;75(13 Suppl):Abstract nr A10. **doi: 10.1158/1538-7445.PANCA2014-A10**

Hendley, AM, Bailey, JM, Alsina, J, Iacobuzio-Donahue, CA, Maitra, A, Reynolds, AB, Leach, SD. p120 catenin: A novel regulator of PanIN epithelial cell delamination in preinvasive pancreatic cancer. [abstract]. In: Proceedings of the 105th Annual Meeting of the American Association for Cancer Research; 2014 Apr 5-9; San Diego, CA. Philadelphia (PA): AACR; ***Cancer Research*** 2014;74(19 Suppl):Abstract nr 66. doi:10.1158/1538-7445.AM2014-66.

Hendley, AM, Provost, E, Blake, D, Roeser, JC, Bittman, RW, Reynolds, AB, Leach SD. Loss of p120ctn in the pancreas results in expansion of ductal epithelium and loss of acinar cells. ***Developmental Biology***, Volume 356, Issue 1, 1 August 2011, Page 169, ISSN 0012-1606, http://dx.doi.org/10.1016/j.ydbio.2011.05.625. http://www.sciencedirect.com/science/article/pii/S0012160611009249.

D. *Other Professional Communications*

Oral Presentations:

p120 Catenin suppresses basal epithelial cell extrusion in invasive pancreatic neoplasia
Selected Oral Presentation
Gordon Research Conference on Pancreatic Diseases
South Hadley, MA, July 23, 2015
***Travel award recipient**

Ablation of p120 catenin in embryonic mouse pancreas disrupts acinar cell, islet, and duct development
Selected Oral Presentation
Mid-Atlantic Society of Developmental Biology meeting
Williamsburg, VA, April 21, 2013

Anti-vif activity by a hammerhead ribozyme expressed from a retroviral vector
Selected Oral Presentation
South Carolina Academy of Science
Columbia, SC, March 20, 2009

Cloning of a Hammerhead Ribozyme targeted to HIV-1 virion infectivity factor
Selected Oral Presentation
South Carolina Academy of Science
Clemson, SC, March 20, 2008

Poster Presentations:

p120 Catenin Suppresses Basal Epithelial Cell Extrusion in Invasive Pancreatic Neoplasia
Selected Poster of Distinction
46th Annual American Pancreatic Association Meeting
San Diego, CA, November 5, 2015
**** Women in Pancreas travel award recipient***

p120 catenin: A novel regulator of epithelial cell delamination in early Kras-driven pancreatic cancer
Selected Poster Presentation
106th Annual Meeting of the American Association for Cancer Research
Philadelphia, PA, April 21, 2015

The cytoskeletal regulator p120 Catenin suppresses epithelial cell delamination in preinvasive pancreatic cancer.
Selected Poster Presentation
TMC DDC 6th Annual Frontiers in Digestive Diseases Symposium
Houston, TX, February 7, 2015

p120 catenin regulates PanIN epithelial cell delamination in preinvasive pancreatic cancer
Selected Poster Presentation
AACR Special Conference: Pancreatic Cancer: Innovations in Research and Treatment
New Orleans, LA, May 19, 2014

p120 catenin: A novel regulator of PanIN epithelial cell delamination in preinvasive pancreatic cancer
Selected Poster Presentation
105th Annual Meeting of the American Association for Cancer Research
San Diego, CA, April 6, 2014

Loss of p120ctn in the pancreas results in expansion of ductal epithelium and loss of acinar cells
Selected Poster Presentation
70th Annual Meeting of the Society of Developmental Biology
Chicago, IL, July 22, 2011

SCIENTIFIC COURSES ATTENDED:

2013: Workshop on Pancreatic Cancer, Banbury Campus, Cold Spring Harbor, NY, USA.

2010: 51st Annual Short Course on Medical and Experimental Mammalian Genetics, The Jackson Laboratory, Bar Harbor, Maine, USA.

TEACHING EXPERIENCE:

Teaching Assistant, Genetics and Medicine: History of Ideas, Johns Hopkins University School of Medicine 2013

Teaching Assistant, Microbiology, University of South Carolina Aiken 2007 - 2009

Teaching Assistant, Fundamentals of Genetics, University of South Carolina Aiken 2006 - 2009

PROFESSIONAL ORGANIZATIONS:

American Association for the Advancement of Science, Member, 2015
American Pancreatic Association, Member, 2015
American Association for Cancer Research (AACR), Member, 2013-Present
AACR Women in Cancer Research (WICR), Member, 2013-Present
Society of Developmental Biology, Member, 2011, 2013
American Society of Human Genetics, Member, 2010
Sigma Alpha Lambda (National Leadership and Honors Organization), Member, 2006-2009
Gamma Beta Phi (National Honor and Service Organization), Member, 2006-2009

LEADERSHIP EXPERIENCE:

2010-2014: Organizer and participant, Institute of Genetic Medicine graduate student recruitment, Johns Hopkins University School of Medicine

2007: Selected, undergraduate student representative for the Department of Biology and Geology, University of South Carolina Aiken
*Served on committee participating in faculty recruitment and selection

SERVICE:

2015: Mentor, 1 medical student, The University of Texas Health Science Center at Houston, Laboratory of Dr. Jennifer M. Bailey
*Mentored student during summer research program supported by NIDDK NIH T35 DK007676-22

2011-2013 Mentor, 3 high school students and 1 undergraduate student, Johns Hopkins University School of Medicine, Laboratory of Dr. Steven D. Leach
*Mentored and instructed students with ongoing projects in the laboratory

2011: Volunteer, Scripture Union Peru and the Peruvian Ministry of Education, Kusi, Peru

2009-2011: Mentor, Incentive Mentoring Program, Paul Laurence Dunbar High School, Baltimore, Maryland

2010: Invited speaker, *"Human Genetics: A Career in Science,"* Beechfield Elementary/Middle School, Baltimore, Maryland

REFERENCES

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